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Genetic Engineering and Biotechnology

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EMERGING TECHNOLOGY SERIES:

GENETIC ENGINEERING AND BIOTECHNOLOGY

1998/3 & 4

CONTENTS

UNIDO's *Emerging Technology Series: Genetic Engineering and Biotechnology* is established as a mechanism of current awareness to monitor developments in the genetic engineering and biotechnology sector and inform governments, industry and academia, primarily in developing countries.

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TO OUR READERS

Globalization, the information society, sustainable development: these are the keywords of the day. But what do all these trends and scenarios mean for the individual? Which criteria will we use to organize our lives in the 21st century? The president of the Club of Rome, Professor Ricardo Diez Hochleitner, takes an optimistic look at the years ahead. The Club of Rome (founded in 1968 as an informal gathering of academics, executives and politicians) has assumed responsibility for the overall concept of "Global Dialogue", a series of events to be held during the Expo 2000 world exposition, whose theme "Humankind—Nature—Technology" provides an excellent opportunity to make clear what sort of potential humanity has at its disposal in order to shape its future and survival based on sustainable development.

The members of the Club of Rome from all parts of the world share the view that the future of humanity is not pre-determined and that current and foreseeable conflicts, crises and catastrophes, which to a certain extent are the result of exaggerated selfishness and mismanagement, can be avoided. Humanity cannot continue to afford wars; in our increasingly tightly knit world, where "distant" conflicts no longer exist, such crises lead to a destruction of both human and natural capital, threatening our very existence. The issue of "governance in the global village" presents humanity with one of its greatest challenges, and is inspiring considerable discussion also within the Club of Rome.

The increasingly powerful information society holds many opportunities for building a better future. Overall, the information society possesses the promise of providing access to education in the broadest sense to all humanity. Therefore, information, education and knowledge ought to become a global public good which, in the long term, will create equality of opportunity and contribute to dismantling dangerous inequalities. Furthermore, improved access to information and its dissemination provides every individual with a voice and the practical possibility of control over the forces that govern them.

There can be no doubt that "globalization" has become one of the trendiest words in fashion as our millenium comes to an end, yet this word does not reflect an inevitable natural phenomenon but rather is sincerely indicative of a process to be structured on a number of differing levels. Recent events have shown us that globalization also affects the foundation of our culture and our living together. In the future, our main concerns will be to deal with, on the global level, four long-term goals of equal inportance: first, environmental sustainability; second, economic competitiveness; third, social justice; and fourth, democracy rooted in the rule of law. While these four goals often stand in contradiction to each other, strategically seen, they are nevertheless interdependent. A partial or total renunciation of any one of these goals would endanger achieving any of the others. Globalization, then, is a process which has to be structured quickly and in a positive way. If this is to be systematically achieved the population of the world will have to assume a high degree of responsibility for a common future. Humanity's future will only be secured if our intercourse with nature becomes more respectful, sparing and sustainable. This will require not only all efforts to make use of technological advances, but will demand that we develop new sustainable lifestyles which, at least in part, will require some material renunciation. Moreover, it will be the affluent societies of the north, in their roles as models, which will have to embrace these sustainable lifestyles.

The Club of Rome sees its main mission in the dissemination of this message. Those readers who wish to begin participating in the Global Dialogue now, may do so via the Expo 2000 website: www.expo2000.de

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A. SPECIAL ARTICLES

AN OVERVIEW OF SOIL REMEDIATION TECHNOLOGIES

by
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Abstract

An overview of technologies deployed in the treatment and containment of contaminated soils is presented. The surveyed technologies were grouped into biological, physico-chemical and thermal treatment methods with an insight into the technology limitations, probable estimate for the costs and some Web locations where such technologies are detailed.

Introduction

Remediation of polluted sites has become a recurring decimal as a result of increasing global awareness and concern over pollution, misuse of natural resources, and the environmental and health effects of past developmental processes. This awareness has led to the enactment of various laws and regulations governing all aspects of the storage, treatment and disposal of liquid effluents and sludge in soil (landfills). Stringent cleanup standards are also being directed to protect the soil vegetative and ecological systems and to prevent contamination of the groundwater of hazardous waste and toxic materials from land spills, leachates from landfills, or leakage from underground storage tanks. These measures are critically influencing the development and use pattern of hazardous compounds and also many companies are trading off plum jobs to cope with the treatment of pollutants to regulatory limits using conventional technologies, such as incineration.

Fortunately the scientific community is pouring out various innovative and cost-effective remedial technologies to bring down the attendant high cost associated with conventional technologies and also reducing the man years required for clean-ups.

Remediation technologies can be categorized into "*in situ*"—a process of effecting treatment in place—or "*ex situ*"—a process of treating the contaminant off-site or on-site. Figure 1 depicts the classification of soil remediation technologies. It should be noted that the technology to be used on any site depends mainly on the properties of the contaminant, the concentration of the waste, the soil types, governmental regulation and climatic conditions, although many seem to be in support of *in situ* techniques because of reduced cost and hazard handling resulting from excavation and handling. However, proponents for using *ex situ* seem to base their arguments that *in situ* technologies can prevent reagents, steam, or bacteria from reaching all the contaminated material and that there is less certainty about the uniformity of treatment because of the inherent variability in soil and aquifer characteristics and difficulty in monitoring progress. They claim that *ex situ* allows homogenization of the contaminated soil and ensures monitoring that the soils are cleaned to regulation limits within a relatively short time.

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**See Annex for activities of the Centre's Remediation Subprogramme.

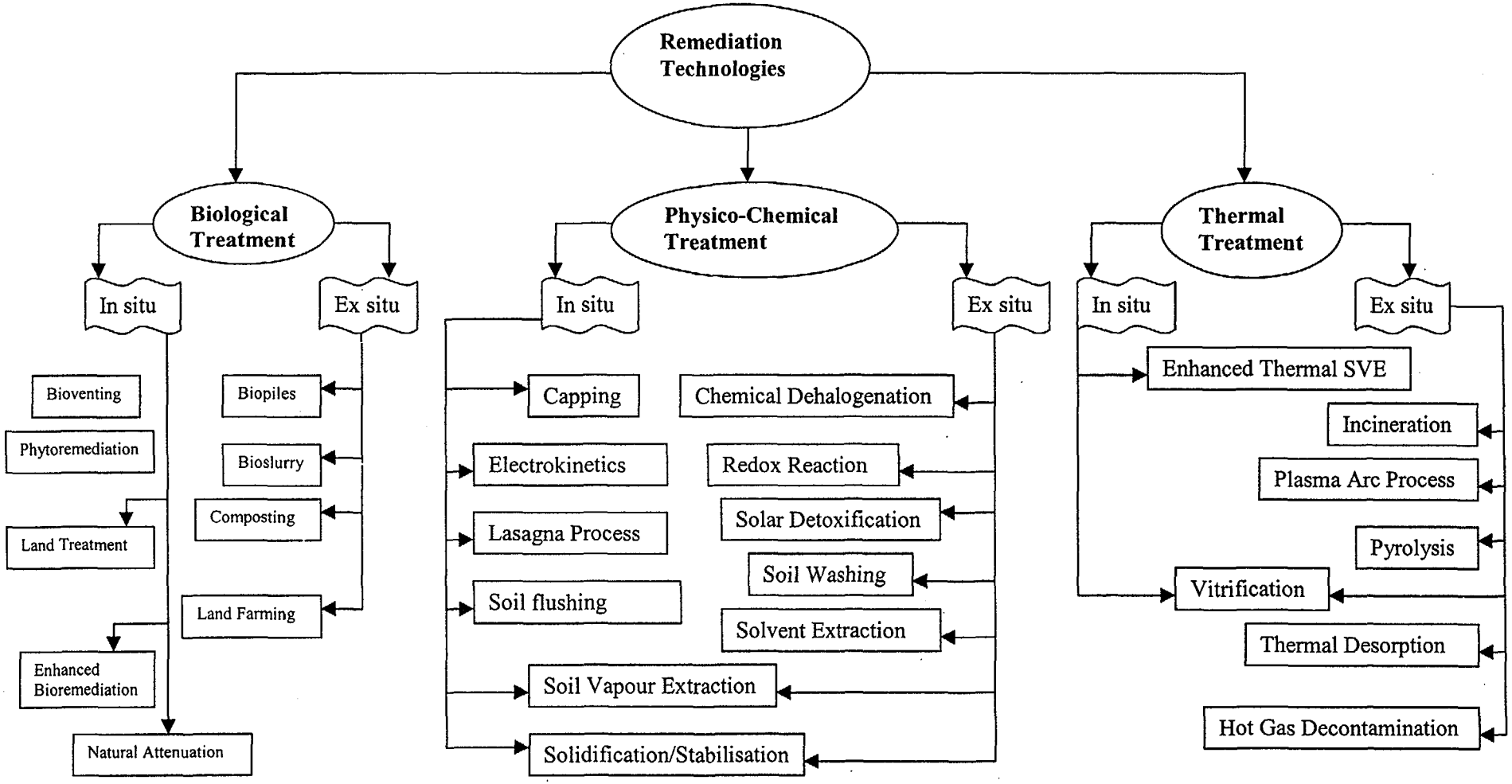

Figure 1 Classification of Soil Remediation Technologies

The basic concepts of remediation technologies

The basic concept of any remediation technologies is to reduce or eliminate deleterious effects of pollutants in the environment. Soil, being a complex matrix, its decontamination is very challenging and requires the analysis and control of a great number of variables to ascertain the best choice that would meet regulatory compliance. There are times that the contaminants may not need any treatment and allow Nature to take its natural course of mitigation (natural attenuation), while in some other cases we may have to choose the best technological option from a train of competing technologies, or a combination of technologies may be preferred in curtailing the menace.

Generally, remediation technologies can be grouped based on their treatment mechanism; biological, physical, chemical, or thermal (see relevant section). These are further subdivided into *in situ* and *ex situ* processes, which are briefly described below. However, we have abridged physical and chemical mechanisms into one group, called physico-chemical, because these two mechanisms normally occur together and overlap in the treatment process. Whereas we listed separately "thermal", because the driving force for the decontamination is heat. An attempt has however been made in presenting a representative list of the various soil remediation technologies currently available.

In situ process

A process is said to be *in situ* if the treatment process is effected while the contaminants are left together with its host, the soil in this case. This process is gaining wider acceptance because of the potentially significant cost savings and the risk associated with excavated soils, although this requires a scheduled monitoring of the treatment process taking place during and after application of the chosen *in situ* technologies. However, it generally takes a longer time to effect treatment to a regulatory level, and there is also the question of uniformity of treatment due to soil heterogeneity. There are also some difficulties such as the monitoring of the process and the induced risk of further contamination of the neighbouring environment.

Ex situ process

Ex situ is the process of removing contaminants along with their host, normally by excavation, to a prepared site made for the treatment. Although this is not easily accepted by host communities and environmental groups (besides incineration), due to material handling and exposure, it generally requires shorter time periods than *in situ* treatment, and there is more certainty about the uniformity of treatment because of the ability to homogenize, screen and continuously mix the soil.

The purpose of the survey

This article aims to present the survey of environmental tools for soil remediation. It presents briefs on established, emerging and innovative bench scale remediation technologies deployed for the decontamination and containment of contaminated soils. The survey took advantage of the "gracious information superhighway", and integrated some Internet sites where these technologies are extended. There is the option to download extended reports on some specific technologies if these Internet sites are visited. Although we have presented available cost estimates to give an insight of the respective technologies as shown in table 1, it should however be taken with caution, as the figures may vary from site to site and by extension from country to country. Besides, the indicated costs were not harmonized as it was only intended to give an insight of the likely costs, which were gathered from a great number of sources, including the cited literature.

Basic parameters of remediation technologies

The selection and use of technologies deployed in the clean-up of contaminated soils are on the increase. We are even witnessing variants of the conventional type, while other innovative and emerging technologies are springing up. The selection of the best remediation technology requires an optimization process where one has to weigh the merits and demerits of all competing technologies. There are a great number of variables to consider in this screening process, which includes the technical aspects (such as soil and contaminant characteristics; time-frame required for the decontamination process), economical aspects (investment cost, maintenance, operations, etc.), together with governmental regulations (regulatory limits, laws) and social aspects (such as public acceptability). An extensive report on cost and performance guidelines which have been prepared by some workers can be downloaded (*Guide*, 1998).

Biological treatments

Biological treatment of waste has been known to man since medieval times. Even farmers and foresters practice it in a limited way by rotating crops and replanting trees. However, due to the huge burden placed on the natural cleansing processes, degradation of many toxic wastes requires some sophisticated engineering techniques to remedy it. One such nature-based engineering technique is called *Bioremediation* (Lee and Banks, 1993; Bragg et al., 1994; Atlas, 1994; Atlas, 1995). It is commonly used for the remediation of organic contaminants and is beginning to be applied to metal remediation, although most applications to date have been at the bench and pilot scale (Schnoor, 1997). Biological treatment exploits natural biological processes that allow certain plants and micro-organisms to aid in the remedial action. These processes occur through a variety of mechanisms, including adsorption, oxidation and reduction reactions, and methylation (Means and Hinchee, 1994).

Biological treatment is therefore a process whereby contaminants in soil, sediments, sludge, or groundwater are transformed or degraded into innocuous substances such as carbon dioxide, water, fatty acids and biomass, through the action of microbial metabolism. The technology can be used either *in situ* or *ex situ,* depending on cost, choice and the required time-frame to meet regulatory levels. There are various innovative biological technologies, such as bioventing, landfarming, composting, enhanced bioremediation, etc. Some of these have been employed in full-scale remediation projects in Australia, Europe and the United States of America.

Limitations:

- Not too efficient for the clean-up of clayey and other low permeability soils;
- The system may require extended treatment time-frames;
- Microbes may often be sensitive to toxins or high contaminants in the soil;
- Possibility of high generation of secondary wastes.

Table 1: A Table of Category, Cost, and Contaminants Amenable to Soil Remediation Technologies.

Treatment Method	Remediation Technology		Category	Cost (US $/ton)	Clean-up time	Contaminants
Biological	Biopiles	(ex)	Established	25 – 75	S	1,2,3,5,6
	Bioslurry	(ex)	Innovative	230 – 270	S to M	1,2,3,5,6,7,10
	Bioventing	(in)	Established	13 –78	M to L	1,2,3,5,6
	Composting	(ex)	Established	250 – 299	M to L	1,2,6
	Enhanced Bioremediation	(in)	Innovative	26 – 104	L	1,2,6,10
	Land farming	(ex)	Established	97.5	M to L	1,2,3,5,6,10
	Land treatment	(in)	Innovative	32.5 – 65	S to L	1,2,3,5,6,10
	Natural Attenuation	(in)	Innovative	10,000/yr	L	1,2,6
	Phytoremediation	(in)	Emerging	NA	L	8
Physico-Chemical	Capping	(in)	Established	175k– 225k/acre	L	1,2,3,4,5,6,7,8,9,10
	Chemical Dehalogenation	(ex)	Innovative	200 - 500	S	1,2,4,5,7
	Redox Reaction	(ex)	Established	195 – 650	S to M	1,2,8,9
	Electrokinetics	(in)	Emerging	65 – 195	S to M	8
	Lasagna process	(in)	Emerging	52 – 117	S to M	1,2,5,8
	Soil Flushing	(in)	Innovative	75 - 210	M	1,2,3,5,6,8
	Soil Vapour Extraction	(in)	Established	13 – 52	M to L	1,2,5,6,
	Soil Vapour Extraction	(ex)	Innovative	<130	M to L	1,2,5,6
	Soil Washing	(ex)	Innovative	137 – 401	S to M	1,2,3,4,5,6,8,9,10
	Solidification/Stabilisation	(in)	Innovative	111 – 194	M to L	3,4,8,9
	Solidification/Stabilisation	(ex)	Established	73 – 85	S to M	8
	Solar Detoxification	(ex)	Innovative	NA	M	1,2,3,4,10
	Solvent Extraction	(ex)	Innovative	150 – 450	S to M	1,2,3,4,5,6,7,10
Thermal	Enhanced Thermal SVE	(in)	Innovative	32.5 – 130	S to M	1,2,6,10
	Incineration	(ex)	Established	220 – 6,000	S to M	1,2,3,4,5,6,7,10
	Hot Gas Decontamination	(ex)	Innovative	NA	S to M	1
	Plasma Arc Process	(ex)	Innovative	750 – 1900	S to M	4,10
	Pyrolysis	(ex)	Established	300	S	1,2,3,4,5,6,7,10
	Thermal Desorption	(ex)	Established	40 – 300	S to M	1,2,3,5,6,8
	Vitrification	(in)	Innovative	300 – 400	S	1,2,3,4,5,6,7,8,9,10
	Vitrification	(ex)	Innovative	NA	S	1,2,3,4,5,6,7,8,9,10

1-SVOCs, 2-VOCs, 3-PAHs, 4-PCBs, 5-Halogenated Organics, 6-Non-halogenated Organics, 7-Dioxins/Furans, 8-Heavy Metals, 9-Nonmetals,10-Pesticides

Biopiles

Biopiles, or engineered biopiles, is a modification of the land farming method of petroleum hydrocarbon contaminants decontamination, and very advantageous in relatively limited landspace and the capturing and treatment of volatile organic compounds. It is a full-scale *ex situ* bioremediation technology in which the polluted excavated soils are stockpiled into a heap within the treatment bed so as to prevent further contamination, mixed with soil amendments, and with a delivery aeration system. The process also consists of an irrigation/nutrient system applied to the treatment heap, while a leachate collection system is used to recycle the collected fluid. Moisture, heat, nutrients, oxygen, and pH are controlled to enhance biodegradation of the contaminants. This process normally reduces the contaminants to carbon dioxide and water within three to six months of operation (*Treatment Technology*, 1999).

Limitations:

- Contaminanted soils should be greater than 200 cubic metres to be advantageous over off-site disposal;
- Relatively large space is needed for the treatment bed;

Bioslurry

Bioslurry is a good option for sites requiring greater process control, more complete degradation, or where the cost of importing compost amendments is prohibitive. The contaminated soils are mixed with water to form a slurry so as to allow contact between the micro-organisms and the contaminants. The slurry is then fed into a bioreactor where a controlled amount of air is allowed for mixing and aeration, and inoculation may be permitted to enhance treatment. Because conditions are optimized for the micro-organisms, slurry processes are faster than many other biological processes. The treated slurry is suitable for direct land application, similar to composted soils.

Limitations:

- Ineffective in the removal of metals;
- Disposition of the treated soil is an important part of bioslurry process costs.

Bioventing

Bioventing is one of the cost-effective innovative approaches in mitigating some of the limitations of the Soil Vapour Extraction (SVE) technology. It is a process whereby air is supplied through injection wells, and in some cases, circulating existing air through vacuum extraction. This, therefore, increases the volatilization of organic contaminants while simultaneously creating a conducive environment for the biodegradation of the less volatile organics. Although many variants of this technology exist, the basic principle is to deliver low and optimized airflow rates to provide enough oxygen to the zone of contamination, and the addition of nutrients to sustain and promote biological degradation of organic compounds by the naturally occurring soil micro-organisms. The optimal flow rates maximize the biodegradation as vapours move slowly through biologically active soil while minimizing volatilization of contaminants (*Treatment Technology*, 1999).

The process is applicable for the removal of organic compounds with moderate to low volatility such as petroleum hydrocarbons, oils and lubricants. Marley and Hoag (1984) demonstrated 99 per cent recovery of gasoline hydrocarbons using this technique.

Limitations:

- Not appropriate for the treatment of metal and inorganic contaminants;
- Soil heterogeneities may affect the effectiveness of the required clean-up level;
- Low permeability soils are difficult to aerate;
- Soils with low moisture content tend to dry out during aeration, limiting biodegradation.

Composting

Composting is an *ex situ* solid phase remediation technology like land farming. Unlike land farming, composting requires thermophilic (55 to 65° C) conditions due to the increased biological activity taking place in the degraded organic waste. In this treatment profile, contaminated soil is excavated and mixed with bulking agents and organic amendments (such as wood chips and vegetative waste) to improve soil texture for aeration and drainage. Proper amendment selection ensures adequate porosity and provides a balance of carbon and nitrogen to promote thermophilic, microbial activity. The system is optimized by maintaining moisture content, pH, temperature, nutrients via irrigation techniques (Bossert and Compeau, 1995; FRTR, 1997) and the carbon-to-nitrogen ratio achieves maximum degradation efficiency. At the end of the process, an organic-rich compost remains. This material can then be placed back onto the contamination site, providing a very fertile soil for revegetation.

The composting process is applicable to soils contaminated with biodegradable organic compounds, heavy oils, PAHs, and munitions wastes such as 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). In fact it was reported that on a field experiment using compost, that TNT reductions were as high as 99.7 per cent in 40 days, while removal efficiencies for RDX and HMX were 99.8 per cent and 96.8 per cent, respectively (FRTR, 1997).

Limitations:

- Off-gases of VOCs may pervade the environment due to excavation;
- Composting results in a volumetric increase in material because of the addition of amendment material;
- Metals may affect the clean-up performance and can be toxic to the micro-organisms.

Enhanced bioremedaition

Enhanced bioremediation, which can also be called biostimulation or bioaugmentation, is a process of increasing the biodegradation rate of contaminated soil by the addition of nutrients and oxygen. The activity of microflora and fauna may be stimulated by circulating water-based solutions through the contaminated soils and/or addition of indigenous/inoculated micro-organisms, engineered microbial species or seeding with pollutant degrading bacteria so as to enhance biological degradation of contaminants or immobilization of inorganic contaminants. Although it could be done in the absence of oxygen, it is more advantageous when oxygen is not limiting so as not to get other persistent by-products, such as vinyl chloride, resulting from the anaerobic degradation of trichloroethylene.

The process could be used to treat soils contaminated with a variety of waste, including petroleum hydrocarbons, solvents, pesticides, wood preservatives, nitrotoluenes, changing of valence state of inorganics and cause adsorption, immobilization onto soil particulates, precipitation, uptake, accumulation, and concentration of inorganics in micro- or macro-organisms.

Limitations:

- Water-based solution circulation may pose dangers to underlying groundwater;

- Clogging may occur;
- Not suitable for low permeability soils;
- High metalic and chlorinated organic concentrations may be toxic to the organisms;
- Ineffective at low temperatures.

Land farming

Land farming is an age-old *ex situ* solid-phase remediation technology for the treatment of petroleum hydrocarbon contaminated soils. It is a technique designed to enhance the microbial degradation of contaminants through periodic tilling to induce aeration, controlled moisture content and addition of nutrients such as nitrogen and phosphorus. Pope and Matthews (1993) gave a relatively standard methodology for this technology. Normally, the contaminated soil is excavated onto a designed lined bed (properly lined to prevent leaching) and mixed with controlled amounts of nutrients and soil amendments such as bulking agents (Duncan et al., 1998; Bossert and Compeau, 1995; Pope and Matthews, 1993; Preslo et al., 1989). Bioaugmentation of microbial culture may also be added to enhance the degradation rate into benign compounds. The process is also subjected to periodic tilling to induce aeration.

Limitations:

- Chlorinated and nitrated compounds may affect contaminant degradability;
- Climatic conditions may affect the length of treatment;
- Volatile organics may off-gas into the surrounding air, thus posing environmental risks;
- Requires large spacious area for treatment;
- Inorganics may impede treatment performance.

Land treatment

Land treatment is an *in situ* technology whereby the contaminated soils are tilled on site and the combined forces of bulk soil interaction, biological activity and climatic conditions effect clean-up.

Limitations:

- There is a limited depth of achievable tilling for treatment;
- Leaching of the contaminants may pose a danger to the groundwater.

Natural attenuation

Natural attenuation or "intrinsic bioremediation" is the process of allowing contaminants in place to undergo degradation and mineralization under natural processes such as dilution, diffusion, dispersion, advection, volatilization, microbial degradation, sorption and chemical reactions within an acceptable time-frame. This technique is used whenever it is envisaged that natural processes will mineralize the contaminants to acceptable regulatory risk levels. The process requires virtually no intervention except monitoring of the mineralization process. Contaminants that can be treated using this technology are mostly volatile organic compounds (VOC) and moderately semi-volatile organic compounds. Fuel hydrocarbon can also be remediated by this technology. It has also been seen to result in changes in some metals, like the reduction of soluble hazardous chromium hexavalent ion to chromium three, which is less hazardous.

Limitations:

- Not viable for high contaminants concentration of more than 25,000 ppm;
- Takes extremely long time-frame to effect clean-up;
- Insufficient microbial activity;
- Potential source of pollution to human and ecological receptors.

Phytoremediation

Phytoremediation is an emerging cost-effective remediation technology for *in situ* vegetative treatment of hazardous contaminants in soils and water, and sometimes in air. This technology makes use of specific plants and planting techniques to accelerate the rate of degradation, removal, transformation, stabilization and destruction of targeted soil, water and even airborne contaminants (Burken and Schnoor, 1996). Although it is best applicable at sites with shallow contamination scenario of organics and metals, some practices now make use of deep-rooted plants such as poplars and alfalfa to attack, mitigate and contain pollutants situated many feet into the subsurface.

The degradative process stimulates micro-organisms through the release of carbon-containing nutrients from their roots. The zone closely associated with the roots of plants, the rhizosphere, has a much higher number of metabolically active micro-organisms as a result of naturally released nutrients which they use for energy and other biological activity. It is this symbiotic relationship between plants and microbes that is responsible for the degradation process. Other known mechanisms whereby plants are able to effect a remediation process are by hydraulical barrier/containment, phytovolatilization, phytoaccumulation and phytodegradation. A detailed bibliography of phytoremediation (about 1,446 citations) has been prepared from peer-reviewed journal articles, presentations and posters from conferences, book chapters, and articles from newspapers and magazines, which are alphabetically displayed or by search engine with given parameters (RTDF, 1999).

Limitations:

- Takes many years of clean-up to regulatory limit;
- May pose contamination of the food chain;
- Limited data to enhance standardization and regulatory acceptance.

Physical/chemical treatments

Physical/chemical treatment uses the physical properties of the contaminants or the contaminated medium to destroy, neutralize, precipitate, dechlorinate, separate, or contain the contamination. It is typically cost-effective and can be completed in short time periods (in comparison with biological treatment). Treatment residuals from separation techniques will require treatment or disposal, which will add to the total project costs and may require permits. Extraction fluids from soil flushing will increase the mobility of the contaminants, so provisions must be made for subsurface recovery.

Capping (landfill cap system)

Landfill capping can be regarded as the most common form of soil-remediation technologies. It is used to cover buried waste materials to prevent contact with the environment and is believed to effectively manage human and ecological risks associated with a remediation site. The design of landfill caps is site-specific and depends on the intended functions of the system. The most critical components of a landfill cap are the barrier layer and the drainage layer. Landfill caps can range from a one-layer system of vegetated soil to a complex multi-layer system of soils and geosynthetics. In general, less complex systems are required in dry climates and more complex systems are required in wet climates. The material used in the construction of landfill caps include low-permeability and high-permeability soils and low-permeability geosynthetic products. The low-permeability materials divert water and prevent its passage into the

waste. The high-permeability materials carry water that percolates into the cap away. Other materials may be used to increase slope stability.

Landfill caps may be temporary or final. Temporary caps can be installed before final closure to minimize generation of leachate until a better remedy is selected. They are usually used to minimize infiltration when the underlying waste mass is undergoing settling. A more stable base will thus be provided for the final cover, reducing the cost of the post-closure maintenance. Landfill caps may also be applied to waste masses that are so large that other treatment is impractical. At mining sites for example, caps can be used to minimize the infiltration of water to contaminated tailings piles and to provide a suitable base for the establishment of vegetation. In conjunction with water diversion and detention structures, landfill caps may be designed to route surface water away from the waste area while minimizing erosion.

Limitations:

- Landfilling does not lessen toxicity, mobility, or volume of hazardous wastes, but does mitigate migration;
- Necessary precautions must be taken to assume that the integrity of the cap is not compromised by land use activities.

Chemical dehalogenation

Chemical dehalogenation, whose two common versions in use are "based catalyzed decomposition (BCD)" and glycolate dechlorination; it is an innovative chemical process used to decontaminate soils contaminated with halogenated organic compounds. This technology, which involves the screening and processing of excavated soil mixed with chemical reagents in a heated (330° C) chemical reactor, has been reported to be very effective for the removal of chlorinated substances, especially polychlorinated biphenyls (PCBs), dioxins, furans, and certain chlorinated pesticides (EPA, 1996).

Limitations:

- High clay and water content may impede treatment;
- It requires sufficient space for screening excavated soil.

Redox reaction technology

Redox reaction technology is a chemical reactive process (involving the transfer of electrons from one compound to another), whereby ozone (O_3), hydrogen peroxide (H_2O_2), hypochlorides, chlorine dioxide, or chlorine compounds are added to the target contaminants which induces a redox reaction that chemically converts contaminants into less toxic, less mobile, inert, or more stable compounds.

Effective for treatment of inorganics, in particular chromium (VI), arsenic, and cyanides, also non-halogenated VOCs and SVOCs.

Limitations:

- Organics may impede treatment performance;
- Suitable for low concentration of inorganics;
- Possibility of a toxic by-product due to incomplete oxidation.

Electrokinetics

Electrokinetic remediation is an emerging technology targeted mainly to the *in situ* removal of heavy metals from a contaminated soil matrix. However, it can also be used for the separation and mitigation of radionuclides and organic contaminants. The basic concept of this technology is ions and water migration in an electrical field. The movement of pore water is termed electro-osmosis while the movement of ions is called electro-migration. Some accounts of this remediation technology have been reported elsewhere (Acar et al., 1993, 1995; Cabrera-Guzman et al., 1990). This technique is mainly targeted at metallic contaminants in the soil matrix and involves the application of direct current potential across the contaminated zone through the use of electrodes in the ground.

Surfactant-Enhanced Electrokinetics is aimed at addressing the limitations of electrokinetics technology by injecting surfactant into the soil at one of the electrodes, which reduces the interfacial tension between the contaminant and the soil matrix so that the surfactant can then extract the organics from the surface of the soils and carry them towards the cathode. Because the direction and rate of flow is controlled, there is maximum contact between the surfactant and the soil particles and hence maximum mobilization of the organics.

Limitations:

- It will not remove organics;
- It is heavily dependent on the soil moisture content.

Lasagna process*

This is an integrated *in situ* soil remediation technology aimed at overcoming the deficiencies of other remedial technologies with regards to effecting clean-ups to regulating and safe levels in sites with low permeability soils (Ho et al., 1995; Lasagna, 1996). The technology combines the effectiveness of electro-osmosis, biodegradation and physico-chemical treatment processes to treat soil and soil pore water contaminated with soluble organic compounds.

The Lasagna concept uses electrokinetics to move contaminants in soil pore water into a treatment zone installed directly in the contaminated area where the contaminants are captured, immobilized or degraded. However, for highly non-polar contaminants, surfactants can be incorporated to solubilize the organics, while for a mixture of organic and metalic wastes the treatment zones can contain sorbents for binding the metals and contain microbes or catalysts for degrading the organics (Ho et al., 1995).

Limitations:

- Neutralizing pH near the cathode and anode;
- Limited knowledge on the treatment chemistry and procedures;
- Possible diffusion from untreated zones during treatment may impede effectiveness of treatment.

Soil flushing

Also known as injection/recirculation or *in situ* soil washing. This is a process of injection or infiltration of a solution into a zone of contaminated soil designed to flush out the contaminants into a zone from where they will be extracted. The technique entails the drilling of injection and extraction wells and the addition of a solution (Roote, 1997). The solutions may consist of surfactants, cosolvents, acids, bases, solvents, or plain water. Normally any variety of configurations of injection wells, horizontal wells, trenches, infiltration galleries and extraction wells may be used to contact the flushing solution with the contaminated zone. It is best applicable to a variety of organic contaminants (non-aqueous phase liquid (NAPL)) in moderate to high permeability soils.

Limitations:

- Method not too efficient for low permeability soils;
- Flushing solution must be contained and recovered from contaminating other subsurface bodies;

*Lasagna process can be carried out by either physical (trapping), chemical (catalysts or reagents), biological (microbes or enzymes) treatments, or a combination of the three methods.

Soil vapour extraction

Soil vapour extraction (SVE) is a well established and widely used remediation technology. It is a cost-effective and efficient technique for the removal of volatile organic compounds (VOCs). The technology can be used for treating contaminants *in situ* or *ex situ*. In *in situ* SVE, a vacuum is applied through extraction wells to create a concentration gradient and a zone of low vapour pressure that induces gas phase volatiles to be removed from the soil through the extraction wells. For *ex situ* SVE remediation, the excavated soil is placed over a network of above-ground piping to which a vacuum is applied to encourage volatilization of organics. The soil piles may be covered with a geomembrane to prevent volatile emissions and to prevent the soil from becoming saturated by precipitation. This technique has an edge over its sister *in situ* method as a result of increased passageways, ability to collect leachate, and more uniform treatment. It involves the pumping of air from the subsurface by using vacuum pumps to withdraw air from the wells. The process is very effective for the removal of NAPL in permeable, relatively homogenous soils.

Limitations:

In situ

- Limitation on mass transfer to the air phase due to low contaminant volatility and/or diffusion-controlled transport have been identified as primary factors constraining efficiency of SVE;
- Performance is also limited by low permeability, high soil water content and heterogeneities.
- Exhaust air from *in situ* SVE system may require treatment to eliminate possible harm to the public and the environment.

Ex situ

- Huge space required;
- Excavation and materials handling may pose hazardous emissions to the surroundings.

Soil washing

Soil washing is an *ex situ* soil remediation technology for the removal of hazardous contaminants sorbed onto the soil matrix. It is a scrubbing technique whereby the excavated contaminated soil is fed into a wash-solution system under pH-controlled chemical additives. This process thus removes and concentrates the contaminants for further treatment. Because the contaminants often bind to silt or clay, the excavated soils must be sifted and separated, thus reducing the volume of contaminated soil that needs treatment.

The technology is designed to remove metals, petroleum hydrocarbons, and polynuclear aromatic hydrocarbons (PAHs) from the soil. Other applications include soils from gasworks, petrochemical plants, coke manufacturers, and iron and steel manufacturing plants and foundries.

Limitations:

- Soil with high humic content may require pretreatment;
- Additional expenses to treat the aqueous stream.

Solar detoxification

This is an emerging and innovative remediation technology for the destruction of a wide range of hazardous organic chemicals in soil and/or water by photocatalytic oxidation or direct thermal decomposition. This process taps photons directly from solar energy or may generate ultraviolet radiation (UV) photons from electric lamps used to promote an oxidative reaction in photocatalytic reactions. A semiconductor catalyst, titanium dioxide (TiO_2), is normally used as the photocatalyst for hydroxyl radical generation. These hydroxyl radicals then react with the contaminant molecules in the waste to effect a breakdown into non-toxic by products such as water, carbon dioxide and an inorganic salt.

It has been reported elsewhere (CMPS&F, 1997) that some research centres in the USA have developed a solar-enhanced thermal treatment for solar detoxification of organic contaminants from both soils and liquid wastes whose destructive and removal efficiency of organics, including PCBs, is of a magnitude of more than 100 over conventional thermal technologies. This treatment effectiveness was corroborated in another field test where contaminants removal was below 5 parts per billion (FRTR, 1997). Nitro-organic compounds, TNT, pesticides, dyes, VOCs, SVOCs, solvents, heavy metals, PCBs, furans, dioxins, polychlorinated biphenyls (PCBs) and a host of other contaminants are amenable to this treatment technology, which is being practiced in most developed countries.

Limitations:

- No adequate information on cost. However, it is believed that the savings in fuel use and lower off-gas generation may make it competitive.

Solidification/stabilization

Solidification/stabilization is an effective method in treating hazardous waste by solidifying or lowering the mobility of the target contaminants, mostly heavy metals. The goal is to prevent contaminated materials from affecting the surrounding environment. To achieve this goal, contaminated soil is mixed (either *in situ* or *ex situ*) with binding materials, such as cement, pozzolans, thermoplastics, fly-ash, lime-kiln dusts and low-cost silicate-containing by-products to produce a stabilized mass (solidification) or less solid material that binds liquids and reduces mobility (stabilization).

Limitations:

- The solidified material may hinder future site use if done *in situ*;
- Environmental conditions may affect the long-term immobilization of contaminants;
- Process is not effective in immobilising organic waste.

Solvent extraction

This is an *ex situ* physico/chemical process of separating hazardous waste contaminants from soil, thereby concentrating the contaminants and reducing the volume of hazardous material that needs to be destroyed. The technology uses an extracting chemical to dissolve a target contaminant from soils in an extractant, and subjects the resultant solution for treatment with recovery of the solvent used. This process produces relatively clean soil or sediment which can be returned to site or disposed of to landfill. In some practices, prior to the solvent extraction, a physical separation technique may be used to screen the soils into coarse and fine fractions, which may enhance the kinetics of the extraction process. This pretreatment technology is very useful in mitigating organic wastes and heavy metal.

Solvent extraction technology can be applied to soils contaminated with volatile and semi-volatile organic compounds and other higher boiling complex organics, such as polynuclear aromatic hydrocarbons (PAHs), petroleum hydrocarbons, pesticides/insecticides, polychlorinated biphenyls (PCBs), dioxins, and pentachlorophenol (PCP).

Limitations:

- Least effective on high molecular weight organic and hydrophilic substances;
- Metals bound with the organics may be extracted, thus compounding treatment options;

- Soil types and moisture content levels will adversely impact process performance.

Thermal treatments

This treatment mechanism is very effective with regards to time-frame for the decontamination of burdened contaminated soil through the application of heat. The processes associated with it may be highly capital-intensive, which may lead to permanent destruction of soil life. The various technologies require off-gas treatment facilities and is effective for a variety of soil contaminants.

Enhanced thermal SVE

Enhanced thermal SVE (often regarded as hot air injection, steam injection, electrical resistance heating or radio frequency heating) is an innovative *in situ* remediation technology of delivering energy to the contaminated zone by hot air, steam, resistive heating or radio frequency so that it heats the contaminated soil, which in turn increases the volatilization rate of semi-volatiles organic compounds from the contaminated soil matrix. The stripped contaminants are brought to the surface via soil vapour extraction technique. Besides VOCs, SVOCs, this technique can be useful for fuel and pesticide soil decontamination.

It has been reported elsewhere (FRTR, 1997) that it would take 9 months to treat a site consisting of 18,200 metric tons (20,000 tons) of contaminated media.

Limitations:

- Hot air injection has limitations due to low heat capacity of air;
- Soil with highly variable permeabilities may result in uneven delivery of gas flow to the contaminated regions;
- Effectiveness of method is greatly reduced by low permeability soils and heterogeneities.

Hot gas decontamination

In hot gas decontamination systems, excavated contaminated soil is subjected to a temperature of about 260° C over a specified period of time. It is a process whereby heated gas is made to thermally decompose or volatilize the contaminants while the off-gases are destroyed in an after burner system thermal oxidizer. It has been reported to be very effective in the treatment of items contaminated with explosives such as TNT, RDX and Tetryl (FRTR, 1997).

Limitations:

- There may be explosions from improperly demilitarized mines or shells.

Incineration

This is one of the most well-known and established remediation technologies for the treatment of a variety of contaminant sources, including explosives. It is a high temperature (870° C to 1,200° C) destructive *ex situ* treatment of polluted soil. It is a process whereby the excavated contaminated soil is fed into the incinerator and the high temperature in the presence of oxygen volatilizes and combusts the contaminants into innocuous substances. Although a variety of designs are available, most incinerator designs are fitted with rotary kiln combustion chambers equipped with an afterburner, a quench tower and an air pollution control system. Removal efficiency of more than 99.99 per cent is feasible, while PCBs and dioxins can be destructively removed to meet 99.9999 per cent levels.

Limitations:

- The treated soil will have zero organic content and cannot support plant life;
- Heavy metals can produce a bottom ash that requires stabilization;
- Volatile heavy metals, including lead, cadmium, mercury and arsenic, may leave the combustion unit with flue gases and require the installation of gas cleaning systems for removal;
- Metals can react with other elements in the feed stream, such as chlorine or sulphur, forming more volatile and toxic compounds than the original species. Such compounds are likely to be short-lived reaction intermediates that can be destroyed in a caustic quench.

Plasma arc process

This fluidic remediation technology utilizes high temperature (10,000° C or even more) pyrolysis, which results from the discharge of a large electric current in an inert gas, to convert hazardous chemicals such as PCBs, pesticides, CFCs and halon gases into innocuous and safe emitted end products. The destructive process is made possible by the conversion of the hazardous substance by the superheated cloud of gas or plasma into atomic forms and subsequent treatment converts the atomic forms into innocuous substances.

In the plasma arc treatment a thermal plasma field is created by directing an electric current through a low pressure gas stream. Plasma arc fields can reach 5,000 to 15,000° C. The intense high temperature zone can be used to dissociate the waste into its atomic elements by injecting the waste into the plasma, or by using the plasma arc as a heat source for combustion or pyrolysis.

Limitations:

- Requires a separate extraction process, such as solvent extraction or thermal desorption to remove the contaminants from bulk solid media such as capacitors or contaminated soil;
- Solid must first be converted to liquid (slurry-like) or gaseous prior to treatment;
- Metals may impede treatment and should be separated for treatment to be effective.

Pyrolysis

Pyrolysis is an emerging *ex situ* remediation technology. It is an anaerobic technique of chemical decomposition whereby hazardous organic compounds are transformed, under pressure and heat, into gaseous components such as methane, carbon monoxide and hydrogen, and a residue of ash and carbon content. This technology mirrors that of an incinerator except for the pressure, oxygen and heat requirements. The technology finds use in the treatment of harzardous organic substances in oily sludges, sediments and soils.

Limitations:

- Inability to attack inorganic contaminants in contaminated sites;
- Possible volatilization of metallic contaminants, which must be taken care of;
- Performance depends on the soil moisture content, which in turn has a high correlation with the cost of treatment.

Thermal desorption

Thermal desorption is an *ex situ* physical removal or separation process of volatile and semi-volatile contaminants which are sorbed to the waste media such as soil, sediment and sludge by heating to temperatures (usually between 170 to 550° C), high enough to volatilize the organic contaminants. This cannot be said to be a stand alone technology, as subsequent remedial treatments of the off-gas (which is normally captured by a carrier gas or vacuum system) to remove particulates and contaminants are necessary for the

transformation into innocuous substances. Wet scrubbers or fabric filters are optimal to remove particulates while contaminants are removed through condensation, followed by carbon adsorption, or they are destroyed in a secondary combustion chamber or catalytic oxidizer such as an after-burner. Thermal desorption may use either direct or indirect heat exchange, and air, or an inert gas to transfer vaporized contaminants from the contaminated medium.

Thermal desorption has been widely applied in the treatment of tar-contaminated soils, refinery wastes, wood-treating wastes, creosote-contaminated soils, hydrocarbon-ontaminated soils, non-halogenated VOCs, SVOCs, PAHs, PCBs, pesticides, mixed (radioactive and hazardous) wastes, synthetic rubber processing waste, and paint wastes.

Limitations:

- Dewatering may be necessary to achieve acceptable soil moisture content levels;
- Highly abrasive feed can potentially damage the processor unit;
- Heavy metals in the feed may produce a treated solid residue that requires stabilization;
- Clay and silty soils and high humic content soils increase reaction time as a result of binding of contaminants.

Vitrification

The underlying principle of vitrification is to subject soil to a sufficiently high temperature so as to cause a melt and form a glass when cooled. This technology can either be carried out *in situ* or *ex situ* and involves the insertion of graphite electrodes into the contaminated encased area at sufficiently close spacing and energized, resulting in high electrical resistive heating (more than 1,700° C) to cause the soil to melt into a molten pool. It is applicable for the treatment of organics, inorganics and radionuclides. The organic contaminants will normally be destroyed while the inorganics will be trapped into the vitrified matrix.

Limitations:

- Vitrification is a destructive process and the soil can no longer support agricultural life;
- The vitrified matrix may hinder future use of the sites if done *in situ*.

Conclusion

We have presented a representative list of remediation technologies currently available for the decontamination of soil and water bodies. We have also presented some indicative cost estimates of these technologies to give an insight of any chosen technology, though with a word of caution. It is hoped that this paper may stimulate scientific interest for a more comprehensive and detailed work on soil remediation technologies.

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Annex

ICS-UNIDO Programme in the Area of Pure and Applied Chemistry. Activities in the Subprogramme of Remediation

The International Centre for Science and High Technology is an institution within the legal framework of UNIDO with headquarters located in Trieste, Italy.

The Centre's mandate relates to the transfer of know-how and technology in favour of developing countries, and is justified by the perception that a competitive industrial technological capability cannot be built-up without adequate scientific knowledge and commitment to a sustainable development approach utilizing new and environment-friendly technologies.

The activities of ICS follow an integrated pragmatic approach which include action-oriented research, short-term exchange between research and technologists in industry, dissemination of scientific and technological information through the creation and management of centres of excellence (focal points), consultancy and advisory services, training courses, scientific workshops, high-level seminars, study tours, fellowships, promotion of training arrangements, publication and editing of frontier issues.

In the present work programme the ICS's activities focus to specific sectors within the area of chemistry, environment, new materials and high technology. In selecting the specific subprogrammes and their related activities, special consideration was given to their relevance in relation to the scientific and technological development of developing countries.

Considering that sustainable development depends upon the harmonization of economic growth and environment conservation and protection, the ICS Area of Pure and Applied Chemistry has identified as priority fields in its work programme the following themes, which are of key relevance to economic and industrial development as well as environmental protection:

Catalysis and Sustainable Chemistry, which is an important scientific and technological area for the development of environmentally friendly chemical processes, which in turn form the basis for cleaner industrial technologies development and are also the key elements for an industrial pollution prevention approach. New, less pollutant processes together with the optimization of existing processes depend to a great extent upon the improvement of catalyst performance in the heavy and fine chemical production lines with a direct impact on the quality and quantity of by-products or waste generated.

Environmentally Degradable Plastics, where the expanding global production and consumption of polymeric materials coupled with increasing public awareness of environmental issues have created serious concern about the problems related to the disposal of plastic waste generated by various sectors of human activity. Besides recycling, re-use, incineration and composting, new technological developments of environmental degradable plastics contribute dramatically to the tackling of the environmental issue in specific sectors of plastics use.

Combinatorial Chemistry and Combinatorial Technologies, which have a strong impact on the development of new chemicals (pharma industries, agro-chemicals, new materials). Developing countries need to get acquainted with and gain expertise in combinatorial technologies to help local enterprises remain competitive and economically viable in the coming decades. Combinatorial chemistry and combinatorial technologies have a potential influence not only on industrial growth, but also on environment protection. In fact, by optimizing industrial processes and production, with the lowering of relevant costs, less amounts of waste and by-products are created.

Remediation Technologies, which are becoming an important and economical way to solve the problem of contaminated and polluted sites, especially in developing countries and economies in transition where the environmental issue has been until recently neglected. New technologies, methodologies and solutions are emerging from various applications and are becoming day-by-day more economically viable and feasible.

Due to this important role of Remediation, ICS-UNIDO has developed a series of activities (events, products and projects) in this field, namely:

Events

Scientific Planning and Coordination Meeting on Bio-Remediation, Trieste, Italy, 20-22 November 1996

Training Course on Soil Environmental Assessment and Bio-Remediation Technologies, Budapest, Hungary, 2-14 June 1997

Training Course on Technological and Economic Aspects of Soil Bio/phyto Remediation, 6-17 October 1997, Plovdiv, Bulgaria

Expert Group Meeting on Environmental Pollution and BATEV in Remediation, Trieste, Italy, 19-21 March 1998

Workshop on Waste Management and Remediation of Polluted Sites for Sustainable Development, Hanoi, Viet Nam, 11-16 May 1998

Training Course on Remediation Technologies: New Trends and Tools for Soil Decontamination, Katowic, Poland, 30 November-5 December 1998

Workshop on Remediation Technologies: Applicability and Economic Viability in Northern Africa and Middle East, Cairo, Egypt, October 1999

Products

A Compendium on "Survey of technologies, applicability, economic viability and main players" is being finalized in cooperation with ECE, which also includes four country reports (Brazil, Egypt, Russia and Viet Nam) on this subject.

Projects

"Integrated Info-package on Remediation Technologies and their Applications" presented at the European Commission within the Leonardo da Vinci programme, it has been developed with institutes from Germany, Austria, Italy, Poland and Slovak Republic.

"Integrated Approach for the Remediation of Polluted Sites: A Tool for Water Resources Protection" to be presented to the 5th Framework programme within the call for Energy, Environment and Sustainable Development, it is being devloped with institutes from Italy, Slovenia, Austria and Germany.

Several proposals have been submitted to ICS for development, among them, the project proposal on:

"Development and Application of Novel Technologies for Oil Treatment and Spent Oil Regeneration in Central and Eastern European Countries", which is being developed in collaboration with Konzeko Ltd. and the Slovak Ministry of Environment.

"Integrated Approach to Solve Urgent Contamination Problems Caused by Industrial Activities in Selected Central and Eastern European Countries", which is being developed in collaboration with the Institute of Chemical Technology, Albania, PROTE Co., Poland, Eko Centrum, Slovak Republic and Slovak Ministry of Environment.

"Monitoring of Pesticide Contamination in the Red River in Viet Nam and China", which is being developed in collaboration with the Center for Environmental Protection and chemical Safety, Viet Nam.

For more detailed information, the reader can visit the ICS Web Page at the following address: http://www.ics.trieste.it

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USE OF BIOCATALYSTS FOR INDUSTRIAL APPLICATIONS*

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Abstract

Enzymes are valuable tools in industrial processes as the reactions they catalyze generally proceed under mild conditions, high selectivity (chemo-, enantio- and regio-selectivity) and are an alternative in solving environmental problems. Advances in screening technology, directed-evolution methods and genetic engineering have greatly increased the availability of various enzymes for their application in laundering, food processing, glucose production and organic synthesis. This review gives a brief overview of the use of enzymes in different industries, such as detergents, food, textiles, pulp and paper, starches and sugars, oils and fats, beverages, backing, leather and fine chemistry.

Introduction

The use of biological systems to catalyze chemical reactions in commercial processes has recently become increasingly practical and affordable in many industries. Enzymes are natural catalysts that can speed up a chemical reaction. The use of enzymes or microbial cells as catalysts is based on their higher capability in carrying out the desired reactions, due to their high specificity, economic advantages or improved environmental impact.

In general, environmental saving by enzymes can be reached in the following ways:

- Enzymes work better at mild temperatures and in mild conditions. They can be used to replace harsh conditions and harsh chemicals, thus saving energy and preventing pollution. No expensive corrosive-resistant equipment need be used.
- Enzymes are highly specific; the production of unwanted by-products is avoided and there is no need to extensively refine and purify the desired product.
- Enzymes can be immobilized and can therefore be reused several times.
- Enzymes can also be used to treat waste consisting of biological material.
- Enzymes themselves are biodegradable, so they are readily reabsorbed back to nature.

For these reasons, enzymes have been commercially exploited in the food, detergent, pharmaceutical, baking, textile and animal feed industries. Industries such as bioremediation and pulp and paper are also growing end-user markets. Today, enzyme industrial production is dominated by deep-tank or submerged-culture fermentation under clean and highly controlled conditions. Enzymes produced by these processes are industrially used in two different ways: as biological catalysts to manufacture other products, such as food ingredients, specialty chemicals, feed additives, etc., and secondly, involving the use of enzymes as end products, as for instance in detergents, diagnostic and laboratory agents [1, 2]. The detergent, food and starch processing industries still account for 70 per cent of bulk enzyme use (mostly hydrolases such as proteases, amylases, lipases and cellulases). Specialty enzymes now account for around 10 per cent of the market and are increasingly used in the development of new drugs and antibiotics, as well as in medical diagnostic and analytical applications.

The sales of the enzyme industry that amounted to US$ 650 million in 1989, increased to US$ 1 billion in 1993 and continues to grow [2]. The most prominent companies that manufacture enzymes are Novo Nordisk and Genecor International. Novo Nordisk, a Danish company, is the biggest worldwide, with about 50 per cent of the total market and is the prime supplier for all applications. The second most important is Genecor International (joint venture of US-based Eastman Chemical Company and Cultor Ltd. in Finland) with less than 20 per cent of the total market. Gist Brocades, a Dutch company, and Pfizer, a US company, are also important producers.

A novel technology, cross-linked enzyme crystals (CLECs(R)), was developed six years ago by Altus Biologics (Cambridge, MA) and was used for customers interested in small-scale experiments. This technology provides a number of advantages, such as high activity and selectivity, ability to function under mild reaction conditions, ease to disposal and stability in different environments [3]. All these advantages make CLEC catalysts extremely useful in organic synthesis. With this promising profile, the company sales were about US$ 10 million in 1998, but a substantial growth of the market is expected in the coming years.

Despite the almost unlimited potential of enzymatic catalysis, the application of enzymes in industrial processes often calls upon properties not found in enzymes isolated from natural environments. In other words, the industrial processes need enzymes that can function efficiently in extreme salinity, pH and pressures, at elevated temperatures, in organic solvents or detergents. Some practical alternatives to improving enzyme stability include the use of immobilized enzymes or the application of CLECs. However, in recent years, several additional developments have revised this perspective of biocatalysis. Naturally occurring microorganisms were identified that proliferate in both extreme conditions and environments (extremozymes) [4]. In addition, certain enzymes from conventional sources were also shown to work under extreme conditions. Because of this there is added incentive to improve and modify biocatalysts from conventional sources and extreme environments through directed and random mutagenesis. At present, for every enzyme used for commercial applications, a more stable version is available [4]. For example, in pulp and paper

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manufacture lipases, xylanases, cellulases and glucanases are used to remove inks and toners from recycled paper. In many instances, enzymes should have a stable function above 70° C and pH 10. Another example is presented in food processing, where enzymes that function at sufficiently high temperatures maintain aseptic conditions.

This review aims at briefly describing the main industrial processes in which enzymes are used as biocatalysts. Table 1 summarizes the applications of enzymes in different industries, excluding organic synthesis and pharmaceuticals, which will be independently summarized in the last part of the paper.

Detergent industry

Biological detergents are common washing agents because they not only wash clothes but also remove organic stains such as grass and blood. Proteases hydrolyze proteins and break them down into more soluble polypeptides of free amino acids. As a result of the combined effect of surfactants and enzymes, stubborn stains can be removed from fibres. Moreover, the trend towards lower washing temperatures has made the removal of grease spots a bigger problem, particularly for cotton and polyester. Some specific lipases are capable of removing greasy stains such as lipstick, frying fats, butter, sauces, etc. [5].

Amylases are used to remove residues of starchy foods (chocolate, mashed potatoes, custards) and for bleach-containing formulations. When a garment made of cotton or cotton blends has been washed several times, the colours become duller. This effect is due to the formation of micro-fibres that become partially detached from the main fibres. Cellulase can degrade these fibres, restoring a smooth surface to the fibre and restoring the garment to its original colours.

Several enzymes have been developed under commercial patent. These are suited to detergent formulations with different pH values, for low wash temperatures or for bleaching. For example, a cellulase complex (Celluzyme®, a Novo Nordisk product [2]) has properties enabling modification to the structure of cellulose fibrils and causes colour brightening, softening and removal of soil particles.

Food industry

Proteases, lipases/estereases, lactases and catalases are the main enzymes used in diary technology. Proteases and lipases are used to intensify flavour and accelerate the ageing of cheese, and lactases (β-galactosidases) to produce low-lactose milk, ice cream and related products for people who have problems of lactose tolerance.

Cheese production is the largest consumer of acid proteases, in the form of milk coagulants or rennets. These enzymes are endopeptidases and have optimal activity at acid pH. Another enzyme used in cheese production is lysozyme, which replaces the use of nitrates to solve the "late-browning" problems in the manufacture of hard cheese. The cause of this problem (irregular hole formation during cheese ripening) was attributed to the contamination of milk raw materials by the heat-resistant and gas-forming organism called *Clostridium tyrobutyricum*. Lysozyme (acting as a natural anti-microbial agent), when added to milk destined for cheese production, is effective in preventing the growth of *C. tyrobutyricum* and retarding the late-browning problem. Recently, modern biotechnology and molecular biology showed a positive impact on the cheese-making process through the generation of different strains of lactic acid bacteria with novel properties [6]. This improvement can be exploited to produce cheese with enhanced features and at a reduced cost.

Another interesting application of enzymes is the enhancement of flavours. Flavours derived from natural precursors with enzymes have high added value because they can be marketed as natural flavours. The flavour development for diary products (cheese, butter, margarine, alcoholic beverages, milk chocolate and sweets) is achieved by selective hydrolysis of fat triglycerides to release free fatty acids, which act as flavours or flavour precursors. To obtain the extensive hydrolysis lipases are used [5].

The use of protease in pet food allows the hydrolysis of minced meat or meat by-products, providing a liquid meat digest that is easy to handle and transport in the processing plant. During this hydrolysis process, peptides and amino acids are produced and they give a savory flavour that pets like. In order to obtain a pure gelatin from scrap meat or hides by the removal of meat and collagen, proteases are used. In comparison with the traditional process, the soaking time can be reduced by half.

In the production of fishmeal, the fish is cooked and pressed in order to reduce its water content before further processing. The press water or "stick-water", containing valuable proteins, is evaporated in multi-step evaporators. However, the viscosity of the product that tends to solidify on the evaporator walls, thus producing less efficient heat transfer, limits the process. At this point the plant has to shut down in order to boil clean the heating surfaces, using an alkaline solution. To avoid this situation, a protein-splitting enzyme is added to the stick-water, decreasing its viscosity and allowing longer times of operation.

Textile industry

In the textile industries the production of fibres from less valuable raw materials, i.e. upgrading the quality of fibres, is an area of increased interest for biocatalysts. Denim and garment washing, biological dyeing and bleaching, enzymatic fibre modification and bio-polishing for finishing garments of cellulosics are considered as the most promising new applications. Some examples are reported below.

In fabrics made from cotton or blends of cotton and synthetic fibres, the longitudinal warp threads are coated with an adhesive substance known as "size". After weaving, the size has to be removed in order to prepare the fabric for finishing. This process, called desizing, may be carried out in the fabric with strong chemicals as acids, bases or oxidant agents. However, amylases are preferred due to their high efficiency and specific action (starch splitting). Amylases bring about complete removal of the size without any harmful effect on the fabric and are harmless to the environment, reducing wastewater in the process [7].

Bio-polishing is an enzymatic treatment for cotton and other natural fibres based on cellulose. The process is used to remove "fuzz", i.e. small fibre ends protruding from the yarn surface and thereby reduces the hairiness or fuzz of fabrics [7]. The cellulases action eliminates pilling and provides better print definition, colour brightness, surface texture and softness, without any loss of absorbency. Two kinds of cellulase are currently available: acid cellulases, which present the highest activity in acid pH (4.5-5.5) and neutral cellulases that are active in the 5.5 to 8.0 pH range. As the enzymes are biodegradable, they are a favourable alternative to many finishing chemicals and resins that are currently used.

Table 1: Commercial industrial application of enzymes

ENZYME	*INDUSTRY*	*APPLICATION*
α-Amylases	Detergent	Remove starch-based stains such as those produced by potatoes, pasta, rice and custard.
	Starch and sugar	Hydrolysis of the interior glucosidic linkages of starch.
	Textile	Removing of starch from woven materials without any harmful effect on the fabric.
	Alcohol	Used for distilling, malt reduction, starch liquefaction and fermentation aid.
	Baking	Flour supplementation and dough improvement.
	Animal feed	Supplementing the animal's own enzymes and improve digestion.
	Brewing	Degradation of starch, protein and glucan.
Amylo-glucosidases	Fruit juice	Improve juice yields, press capacity and the clarification process.
	Alcohol	Used for starch saccharification and malt reduction.
β-glucanases	Wine	Improve the clarification process.
	Brewing	Improvement of filtration, aid the replacement of malt with barley.
	Animal feed	Degradation of feed components for improvement of feed utilization and nutrient digestion.
β-galacto sidases	Food	De-lactose milk products for lactose-deficient population; prevention of lactose crystallization; sweetness improving.
Catalases	Textile	Polishing of cotton fabrics.
Cellulases	Detergent	Brighten and soften the fabric, and release particles of dirt trapped in the fibres
	Textile	Stone-wash of denim garments and bleach clean-up.
	Paper and pulp	Recycling and processing of office paper; production of softer tissue product.
	Fruit juice and wine	Improve juice yields and colour extraction; help for crushing of wine grapes
	Brewing	Improve the strength of gluten and quality of final bread
Dextranases	Health care	Degradation of dextran and inhibition of bacterial growth in the oral cavity.
Esterases	Oil and fat	Modification of fats.
Invertases	Sugar	Soft-centred candies: saccharose hydrolysis to glucose and fructose.
Glucose oxidases	Baking	Replace the use of chemical oxidants to strengthen the gluten for bread making and achieve better bread quality .
Glucose isomerases	Starch and sugar	High fructose syrups: isomerization of glucose.
Lipases	Detergent	Break down fats, oils and greases removing stains based on salad oils, butter, fat-based sauces and soups, and certain cosmetics such as lipstick.
	Paper and pulp	Enzymatic pitch control: triglycerides are hydrolyzed to less sticky and more hydrophilic components.
	Oil and fat	Modification of fats, manufacture of fatty acid amides, ester synthesis and production of high value chemicals.
	Food	Development of flavour and improvement in the aging of cheese.
	Leather	Degradation of fat.
Pectinases	Fruit juice and wine	Improve juice yields and press capacity; improve the clarification and filtration juices and prevent jellification. Improve wine quality and extract flavour compounds in the winemaking process.
Peptidases	Food	Development of flavour.
Phytases	Animal feed	Reduction of phosphorous discharged to the environment from animal production.
Proteases	Detergent	Remove stains caused by proteins such as blood, grass, egg and human sweat
	Food	Development of flavor, gelatin production, viscosity reduction of fish stick waters. Improvement of nutritional and functional properties of animal and vegetable proteins.
	Animal feed	
	Leather	Removing of hair and bating.
Pullulanases	Starch and sugar	Help the saccharification process; hydrolyze of glycosidic links.
Rennets	Food	Cheese manufacture (coagulation of casein).
Xylanases	Paper and pulp	Reduction of chlorine consumption in pulp bleaching processes.
	Baking	Improve the strength of the gluten, dough machinability and the quality of final bread.

Cellulase is used in stone washing of denim jeans. In the traditional stone-washing process, the blue denim was faded by the abrasive action of pumice stones on the garment surface. Nowadays, the cellulase works by loosening the indigo dye on the denim in a process called "bio-stoning". The use of fewer stones results in less damage to the garments, less wear on machines and less pumice dust in the laundry environment.

Another process in which enzymes play an important role is the bleaching process. Natural fabrics are normally bleached with hydrogen peroxide before dyeing. Bleaches are highly reactive chemicals and any peroxide left on the fabric can interfere with the subsequent step. The traditional method of "bleach cleanup" is to neutralize the bleach with a reducing agent, but the dose has to be precisely controlled. Catalases are a cleaner option, because they are capable of breaking down hydrogen peroxide into water and oxygen.

Pulp and paper industry

For a very long time the pulp and paper industry has been a target for environmental groups all over the world. The legislation requirements have changed and today environmental awareness is well established and environmental protection is a priority. The production of totally chlorine-free pulps increases every year. The xylanase enzyme is used as a bleaching catalyst during pretreatment in the manufacture of bleached pulp for paper. After kraft cooking, xylan precipitates onto the pulp fibres. This re-precipitated xylan covers some of the lignin on the fibres. A xylanase can facilitate the subsequent bleaching by opening up the structure (very selective partial hydrolysis) so the bleaching chemicals can work more efficiently on the remaining lignin. This enzymatic process reduces the use of bleaching chemicals (chlorine chemicals) and increases the brightness of kraft pulp [8].

Enzymes are also used to modify the pulp properties, such as improved fibre flexibility and fibrillation, improved drainage in recycled fibres and the removal of xylan [9]. Pulp fibrillation by cellulases is identified as a means to enhance strength properties in non-wood pulps. Cellulases are used to separate and rearrange paper fibrils, thereby improving the strength of paper by increasing fibre contact and bonding. Xylanases can remove xylan from pulp without affecting other components. This is important in preparing dissolving pulps for rayon manufacture or for recovering hemicellulosic sugars while leaving the cellulose intact. Drainage rates determine the loss of water during the paper formation. Recycled fibres tend to have much lower drainage rates than virgin fibres, therefore, paper machines designed for work with virgin fibres have to operate at lower rates with recycled fibres. Enzyme treatments with cellulases and xylanases can improve the drainage rates of recycled fibres.

Enzymatic pitch control is an example of an application where the environmental benefits are limited, as the normal pitch control does no include any harsh chemicals. "Pitch" is a term used to collectively describe the hydrophobic components of wood (triglycerides and waxes). Pitch and deposit problems are common in paper mills. These problems appear as sticky deposits in the paper machines and can cause holes and spots in the final paper. Lipases hydrolyze up to 90 per cent of triglycerides in the pitch into glycerol/monoglycerides and fatty acid, components which are far less sticky and more hydrophilic (easy to wash) [5].

Following legislation requirements, old newspapers are recycled and processed in de-inking plants. These fibres are again used for newsprint. Another type of paper recycling is mixed office waste, which includes every type of paper used in offices (newspapers, unbleached papers, laser copy paper, etc.). This paper waste contains lignin that can cause darkening of pulp during traditional alkaline de-inking. Cellulases allow the de-inking of the pulp to a high quality without the subsequent bleaching stage. In this process, the enzyme does not affect the ink particles, but works on the cellulose fibres that hold the ink onto the fibres. As a result of the enzymatic action, the toner or ink particles fused to the paper become more loosely attached and are more exposed to mechanical shear forces. Moreover, the toner or ink particles start to be more hydrophobic and easier to float for removal with a fibre-water suspension. The environmental benefit of the enzymatic process in mixed office waste de-inking is that no subsequent bleaching is required to obtain an acceptable brightness.

Chemically pulped fibres are more susceptible than mechanically pulped fibres. This is important because mechanical fibres contain a lot of ligin and are therefore more resistant to the cellulases. Another approach to enzymatically enhanced de-inking is to attack the ink itself. Alkaline lipases can facilitate the removal of lipid/based offset printing inks.

Starch and sugar industry

Starch is the main storage of carbohydrates in plants. Its major commercial source is corn, but wheat starch, potato starch, and starches of other cereal grains are becoming significant. Starch and starch derivatives, such as maltodextrins, corn syrups, and modified starches are used as a functional ingredient in many foods. The main use of processed starch is in the production of glucose, which is subsequently used to produce crystalline dextrose, dextrose syrups or high-fructose corn syrup (HFCSs). The HFCSs act as substitute for ordinary sugar in food and beverage processing, such as soft drinks, meats, baked products, ice cream, sauces, baby food, etc.

There are three basic steps in the enzymatic starch conversion to fructose: liquefaction, saccharification and isomerization [10] (see figure 1). In starch liquefaction, a concentrated suspension of purified granular starch is converted into a soluble solution, a shorter-chain-length dextrin. The enzymes are added at the beginning of the heating period so that starch hydrolysis begins at the moment of gelatinization. By using thermostable alpha-amylase (endo-hydrolysis of 1,4-glucosidic linkages), maltodextrins are obtained which contain different oligosaccharides and dextrins. The maltodextrins are widely used in the food industry as thickeners and also as additives for the drying of very hygroscopic materials. The liquefaction is performed at 105° C and with pH of 5.8-6.5. This conduces the replacement of the alpha-amylase with a mixture of enzymes from *B. stearothermophilus* (thermostable) and *B. licheniformis* (for lower pH). Another engineered protein of the *B. licheniformis* alpha-amylase resulted in an enzyme able to operate at lower pH and higher temperatures. Thus, the engineered amylase is a more robust enzyme that increases the range of viable plant-operating parameters.

The aim of saccharification is to remove single glucose residues from a soluble oligosaccharide by using glucoamylase (exo-enzyme, which splits glucose from the non-reducing end). The enzyme used is isolated from *Aspergillus niger*, which has a pH optimum near 4.2, is extremely stable at 60° C and is produced at high levels by the industrial fermentation process. However, since the substrate is a mixture of both amylase (1-4 linkages) and amylopectin (branched 1-6 links), during saccharification, it requires a

blend of enzymes containing both glucoamylase and pullulanase, the latter breaking the 1-6 linkages efficiently.

In the third step, isomerization, the glucose isomerase converts glucose to its sweeter isomer, fructose. The use of an immobilized enzyme allows a continuous process and avoids the introduction of the enzyme into the product. If the starch is going to be used in the production of maltose instead of fructose, then saccharification is done using β-amylase (exo-enzyme, which splits maltose from non-reducing end) and pullulanase to obtain extra high maltose syrups.

Figure 1. Current industrial practice for starch processes. The term DS refers to the percentage of starch or glucose dry solids suspended in the slurry DE, the dextrose equivalent, is an indication of total reducing sugars as glucose percentage. Un-hydrolyzed starch has a DE of zero and D-glucose has a DE of 100

Starch slurry
(pH 5.8-6.5, 35% DS, 95-105° C)

LIQUEFACTION
Thermostable α-amylase

Liquefied starch
(DE 5-10)

SACCHARIFICATION

β-amylase
Pullulanase
(pH 5.0-5.5, 55° C)
Glucoamylase
Pullulanase
(pH 4.0-4.5, 30% DS, 60° C

Glucose syrups (DE 95-96) | Extra high maltose syrups (DE 45-60)

ISOMERIZATION
Glucose isomerase
(pH 7.0-8.5, 45% DS, 55-60° C)

D-fructose

The wine and fruit juice industry

All types of fruit and berries contain varying amounts of pectin, which acts as a kind of glue holding plant cell walls together. The presence of pectic substances in fruit juice causes an increase in the fruit juice viscosity, thus preventing the filtration and concentration processes. To overcome these problems, pectinases are added to the pulp during the clarification process, as they produce a rapid reduction in viscosity as well as the flocculation of the present micelles (to be separated by filtration or sedimentation). Pectic enzymes are also applied to improve fruit juice extraction. Complete depectinization and de-starching facilitate pressing and ensures high yield [11]. During the maceration and solubilization of fruit tissues, pectic enzymes are used to retain the integrity of the cell wall. Recently, technological innovations introduced the application of pectic enzymes in immobilization supports and continuous-flow systems [11].

In some cases, the addition of cellulases or other enzymes may lead to improved juice yields and better colour extraction. Certain fruits, like pears, are rich in the polysaccharide araban and may require the addition of extra arabanase to the clarification step. In the citrus industry pectolytic enzyme preparations are used in the pulp wash process to reduce viscosity in order to avoid jellyfication of pectin during concentration. Fungal enzymes are now available which show pectolytic activity in acidic juices, such as lemon (pH 2.2-2.8). Their use enables clarification without the need of preservatives.

In the wine industry, the enzymes maintain their activity over longer periods compared to the juice industry. Side activities that can be beneficial for fruit juice processing can be less desirable for winemaking, as they may negatively influence wine quality during storage. Specific enzyme preparations for winemaking have been developed to improve wine quality while at the same time providing the desired technological advantages. Some enzymes used include: beta-glucanase to help the clarification process; cellulase for crushing of wine grapes; a combination of cellulase and pectinase derived from selected strains of *Aspergillus niger* to improve clarification and storage stability etc.

Oils and fats industry

The use of enzymes in the oils and fats industry is new, providing several solutions to both the industry problems and the key to produce novel oils and fats. Lipases can catalyze reactions under mild conditions (i.e. the industrial hydrolysis of fats and oils or the manufacture of fatty acid amides), permitting high specificity; they can therefore be used to obtain high-value chemicals for food and industrial uses at competitive production costs. For example, cocoa butter fat required for chocolate production is often in short supply and the price can fluctuate widely. However, palm oil can be upgraded in a reaction with stearic acid using enzymatic interestification (lipases), with similar properties to cocoa butter [5]. Another example is the use of lipases to enrich polyunsatured fatty acids (PUFAs) from animal and plant lipids. Free PUFAs and their mono- and diglycerides are subsequently used to produce a variety of pharmaceuticals (anti-inflammatories, thrombolytics, etc.) [5].

Esters are used as surfactants in cosmetic products, and also in the production of flavours and fragrances. Traditionally, the production of fatty esters has been carried out by chemical catalysis. However, undesirable side-reactions occur, with poor product yield or hazardous by-products. The specificity of the lipase enzyme reaction results in esters without unwanted by-products.

Animal feed industry

The animal feed industry has some compounds, such as β-glucans, which create a viscous mixture after being solubilized. This leads to poor digestion by animals, characterized by poor absorption and low rate of nutrient uptake. β-glucanases can be added to break down these NSPs. It liberates starch and protein masked by the cell structure in the cereal, leading to an increase of metabolizable energy and protein utilization.

In almost all plant materials used for animal feed, a large part of the mineral phosphorus is bound in the form of phytic acid, which cannot be degraded by monogastric animals. Phytic acid complexes iron and zinc ions, making these metal ions unavailable to assimilation by the animal. For this reason, feed producers have to add inorganic phosphate to the feed as a supplement. The addition of phytase into the animal feed allows animals to digest phytic acid. As a consequence, the

assimilation of metal ions by the animal is no longer affected, and it is possible to reduce both the phosporus content added in the feed and the levels of phosphate pollution to the environment [2].

Alcohol industry

The production of fermented alcoholic drinks is developed from starch-containing raw materials. Starch is composed of long chains of glucose molecules that have to be broken into smaller fermentable molecules which the yeast can transform into alcohol. Enzymes can carry out this process in two stages—liquefaction and saccharification. During the starch liquefaction alpha-amylases are used to break down the gelatinized starch into short molecular fragments (dextrins). In the saccharification step, an amylo-glucosidase is used to attack the starch molecules and dextrins. This enzyme is capable of achieving the complete degradation of starch into fermentable sugars (glucose).

In this way, enzymes replace large quantities of malt (the traditional provider of enzymes) and are much easier to handle and store. Extremely thermostable amylases are available with better activity at the low pH values found in the mash and can be used in the starch liquefaction at 100° C long after enzymes from malt have been destroyed. In addition, industrial enzymes are supplied with a uniform and standardized activity.

Brewing industry

In the traditional process to obtain beer, malt acts both as a raw material providing starch and protein and as a source of enzymes. Considerable savings can be made by replacing at least part of the malt with industrial enzymes and unmalted cereals, such as barley. Unmalted barley containing beta-amylase, glucanases and proteases can replace large amounts of malt.

In their natural form, starch-containing cereals are highly resistant to enzymatic attack. In order to break down this resistance, they are boiled before being added to the malt mash. The boiled (gelatinized) cereals are very viscous and difficult to handle and need to be thinned (liquefied). This is done using a thermostable alpha-amylase.

The slow filtration is often a problem due to the presence of certain polysaccharides, mainly beta-glucans and pentosans that increase the viscosity of the wort. These substances can also cause problems in the final beer filtration process by forming a layer of gel, which blocks the tiny holes of the filters. A single solution is to break down the beta-glucans using a beta-glucanase added during the mashing or at the start of the fermentation process.

During the first stage of fermentation, the yeast forms alpha-acetolactate and is slowly converted into diacetyl (unpleasant flavour). An alpha-acetolactate decarboxylase is used to reduce the production of diacetyl and thereby makes it possible to reduce the beer maturation time. The enzymes break down the alpha-acetolactate directly to acetoin (neutral flavour).

Baking industry

The dough for white bread, rolls, buns and similar products consists of flour, water, yeast, salt and other ingredients such as sugar and fat. Flour consists of gluten, starch, non-starch polysaccharides, lipids and traces of minerals. Amylases can degrade starch and produce small dextrines for the yeast to act upon. Gluten is a combination of proteins, which form a large network during dough formation. It is this network that holds the gas during dough proofing and baking. Enzymes such as hemicellulases or xylanases can improve the strength of the gluten, thus improving the quality of the finished bread.

Alpha-amylases can be used as enzyme supplements. This enzyme degrades the damaged starch in wheat flour into small dextrines, thus allowing yeast to work continuously during dough fermentation. The result is the improvement of bread volume and crumb texture and the development of baked flavour. Also, certain types of pentosanase or xylanase are added to the bread to improve dough machinability, yielding a more flexible, easier-to-handle dough.

Chemical oxidants (bromates, ascorbic acid) have been used to strengthen the gluten for bread making. Oxidative enzymes such as glucose oxidase can partially replace the use of these oxidants and achieve better bread quality.

Leather industry

Hides and skins contain proteins and fat in the collagen fibres. Before the hides and skins can be tanned, these substances must be partially or totally removed. The first treatment is soaking. This step serves to remove the common salt and free the hide from blood and dirt. At the same time non-fibril proteins which hold the fibres together have to be eliminated. Proteolytic enzymes facilitate both the emulsification of natural fat by hydrolyzing the wall of the fat cells, and the soaking operation [12].

In enzymatic unhairing, lime and sodium sulphide are used. These dissolve the hair and open up the fibre structure. Proteases assist in the hair removal and are active to pH 12-13, as in the liming process. The treatment period for enzymatic hair can be substantially reduced if the enzyme solution is fed from the flesh side under pressure. The sulphide and lime requirements can be reduced by as much as 40 per cent (while maintaining the same liming time) due to the use of proteases. Another advantage is to avoid the use of amines, which can be converted into carcinogenic compounds.

Lipases specifically degrade fat and do not damage the leather itself. Lipases represent the method of removing fat in the degreasing process with the lowest environmental impact. For bovine hides, lipases allow tensides to be completely replaced. For sheepskins, the use of solvents is very common, but it can also be replaced by lipases and surfactants. In addition, to make the leather pliable, the raw material requires an enzyme treatment before tanning. This is called bating, by this treatment certain protein components are dissolved and can be washed away. Today, pancreatic-bating enzymes are used in combinations with neutral and alkaline bacterial and fungal proteases. Such combined products have the advantage of allowing the joint performance of deliming (elimination of the lime) and bating.

Fine chemical industry

There is a growing interest in the industrial use of enzymes for synthesizing organic chemicals. An important area is the development of modern drugs and agrochemicals, which requires increasingly enantiomerically pure intermediates. In addition, environmental concerns and cost pressure make it difficult to accept unwanted chemical by-products or stoichiometric reactions. Stereo-selective enzymes are suitable for the synthesis of optically active compounds, which require high enantiomeric purity.

The majority of enzymatic reactions involve the hydrolysis of esters and amides or esterification reactions (involving 40 per cent of the biocatalysis literature) [13].

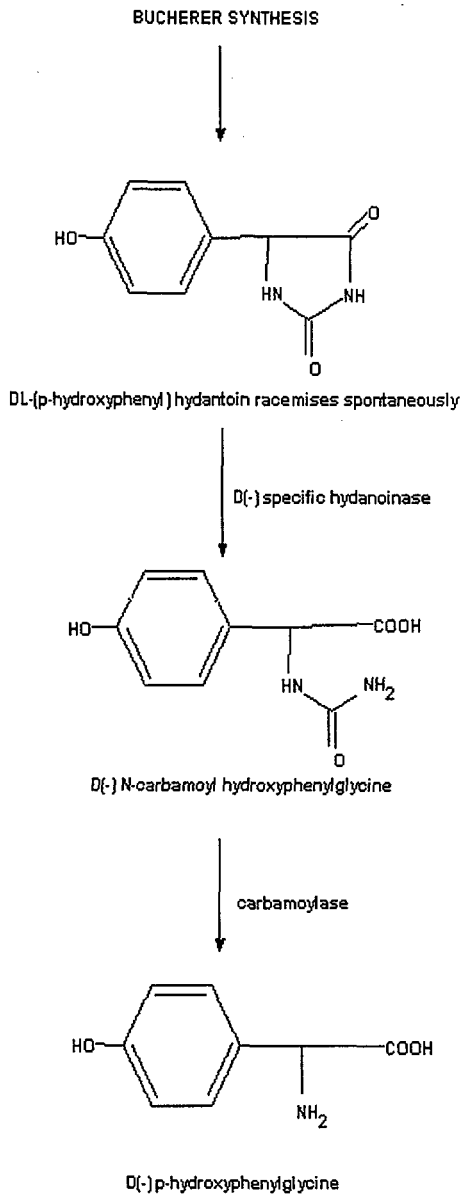
There are also, in minor proportions, other hydrolytic procedures, such as hydrolysis of nitriles, epoxides, etc. (5 per cent). The reduction of carbonyl compounds is well known (10 per cent) and oxidative biotransformations also make up 10 per cent of the current literature. Reduction of carbon-carbon double bond, carbon-carbon-forming reactions, such as cyanohydrin formation and the synthesis of poly-hydroxilated compounds, make up a small percentage of the literature [13]. Table 2 summarizes a wide range of these reactions catalyzed by enzymes [2,5,13-18]. In the following paragraphs, three relevant examples that imply environmental improvement will be presented.

Biocatalysis products for the synthesis of β-lactam antibiotics

The 6-aminopenicillanic acid (6-APA) and 7-amino-deacetoxycephalosporanic acid (7-ADCA) are key intermediates for the production of semi-synthetic penicillins and cephalosporins, respectively. Some years ago 6-APA and 7-ADCA were usually produced by a chemical splitting procedure of penicillin (Pen) or cephalosporin (Ceph). The chemical method is characterized by complicated technical requirements and the use of noxious chemicals (PCl_5), causing environmental pollution as well as impurities in the product [14]. An alternative to the chemical process is the enzymatic catalysis using penicillin amidase (PA) for the biocatalytic production of 6-APA on industrial scale quantities. The process has two fundamental parts. First, the enzyme catalyzes the hydrolysis of Pen and generates 6-APA, which contains the strained β-lactam ring that generates antibacterial activity. The second part is the side chain, D-(-)-hydroxyphenylglycie (HPG). Although total syntheses of HPG are well established, the enantiomeric resolution of this compound is an expensive procedure [2].

A new procedure, utilizing a carbamoylase and hydantoinase enzyme has been introduced (see figure 2 [2]). This method utilizes the Bucherer synthesis to synthesize a racemic mixture of hydroxyphenyl hyndantoin. The hydantoin is then cleaved by the hydantoinase, which is stereoselective for the D-(-) enantiomer, generating D-(-)-N-carbamoyl HPG. This step is followed by further hydrolysis using a carbamoylase producing D-(-)-HPG.

Figure 2. Enzymatic method for D(-) HPG production (100 per cent theoretical yield)



Acrylamide production

The conventional process for the synthesis of acrylamide involves copper-catalyzed hydration of the nitrile. However, this process presents environmental problems, by the formation of many different by-products. To avoid this problem, a new enzymatic process has been designed to produce acrylamide from acrylonitrile using a nitrile-hydrating enzyme (see figure 3 [15]). *Rhodococcus rhodochrous J1* is used as a third-generation catalyst for the acrylamide production and in the industrial production of nicotinamide from 3-cyanopyridine. This strain micro-organism shows a low activity towards acrylamide, and due to its high activity, selectivity and stability, has allowed the establishment of a clean, simple and rapid process without the formation of unnecessary by-products.

Manufacture of aspartame

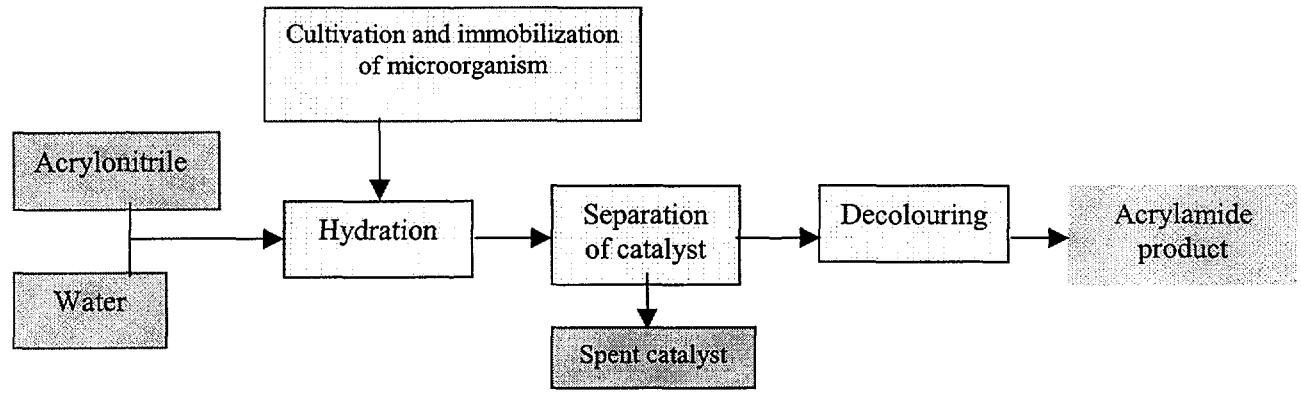
The manufacture of aspartame can be achieved by the synthetic use of the hydrolytic enzyme [14]. Thermolysin catalyses peptide condensation between N-protected L-aspartate and the L-enantiomer of racemic phenylalanine (figure 4). The remaining D-phenylalanine forms an insoluble salt with the dipeptide product. This drives the reaction in the direction of synthesis, contrary to the predicted direction of the equilibrium in the aqueous phase. The D-phenylalanine methyl ester is recovered, racemized and recycled.

Enzyme-catalyzed peptide synthesis offers many advantages to the synthetic chemist, including the absence of racemization and minimal protection and activation requirements [18]. These advantages are rarely exploited in preparative synthesis because of limited catalyst stability. One possibility for the chemical industry is the use of cross-linked enzyme crystals (CLEC ®) of thermolysin. It retains catalytic activity even under harsh conditions (elevated temperatures, organic solvents), indicating that they can be effective in many syntheses.

Table 2: Examples of large-scale biocatalytic processes in chemical manufacture

Process	Catalyst	Product
Hydrolases	Amyloglucosidase	Glucose
	Nitrile hydratase	Acrylamide
	Nitrile hydratase	Nicotinamide
	Penicillin amidase	6-Apa
	D-hydantoinase	D-amino acids
	D-carbamoylase	D-amino acids
	Epoxide hydrolase	(+)-disparlure
	Epoxide hydrolase	R-Nifénalol ®
Resolution	Hydantoinase	4-Hydroxyphenylglycine
	Pseudomonas sp.	Cysteine
	Dehalogenase	(S)-2-Chloropropionate
	Lactonase	Panoyl lactone
	Lipase	N-acylated amine
	CLEC-lipase	S-ibuprofen, S-naproxen and (-)-menthol
	Lipase	Catechin, galangin, quercetin and other flavonoids
Oxidation	Sorbitol dehydrogenase	L-Sorbose
	Dioxygenase	*Trans*-4-hydroxy-L-proline
	Dioxigenase	*Cis*-3-hydroxy-L-proline
	Mono-oxygenase	Optically active lactones
	Mono-oxigenase	(R)-(+)-lipoic acid
	Pseudomona putida	Pancratistati acid and *cis*-chrysanthemic acid
	Mono-oxygenase	Optically active sulfoxides
Reduction	β-Ketoreductase	Carnitine
	aldehyde reductase	(R) and (s)-4-chloro-3-hydroxybutanate ethyl esters (chiral alcohols)
	baker's yeast	(S)-alcohols
Isomerization	Glucose (xyl) isomerase	Isoglucose
C-C synthesis	Pyruvate decarboxylase	Phenylacetylcarbinol
	Tyrosine phenol lyase	L-Dopa
	(R)-oxynitrilase	Optically active cyanohydrins
	(S)-oxynitrilase	Polyhydroxy compounds (as tetrol)
	aldolases	
Achiral precursors	Fumarase	Malate
	Aspartate ammonia lyase	Aspartate
Peptide synthesis	Thermolysin	Arpartame
	Trypsin	Insulin
Glycosyl transfer	Cyclodextrin glucanotransferase	β-Cyclodextrin

Figure 3. Microbial processes for the production of acrylamide. The nitrile hydratase and amidase reactions involved in the acrylonitrile formation are illustrated



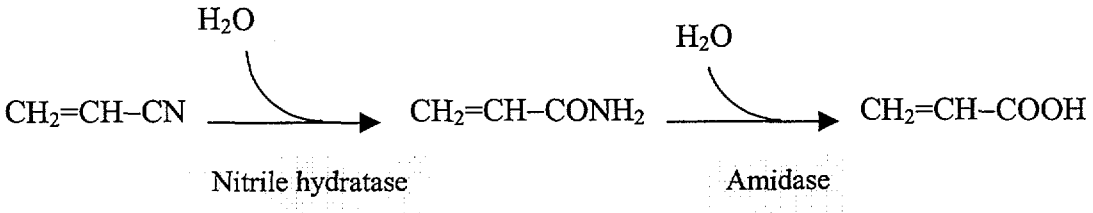
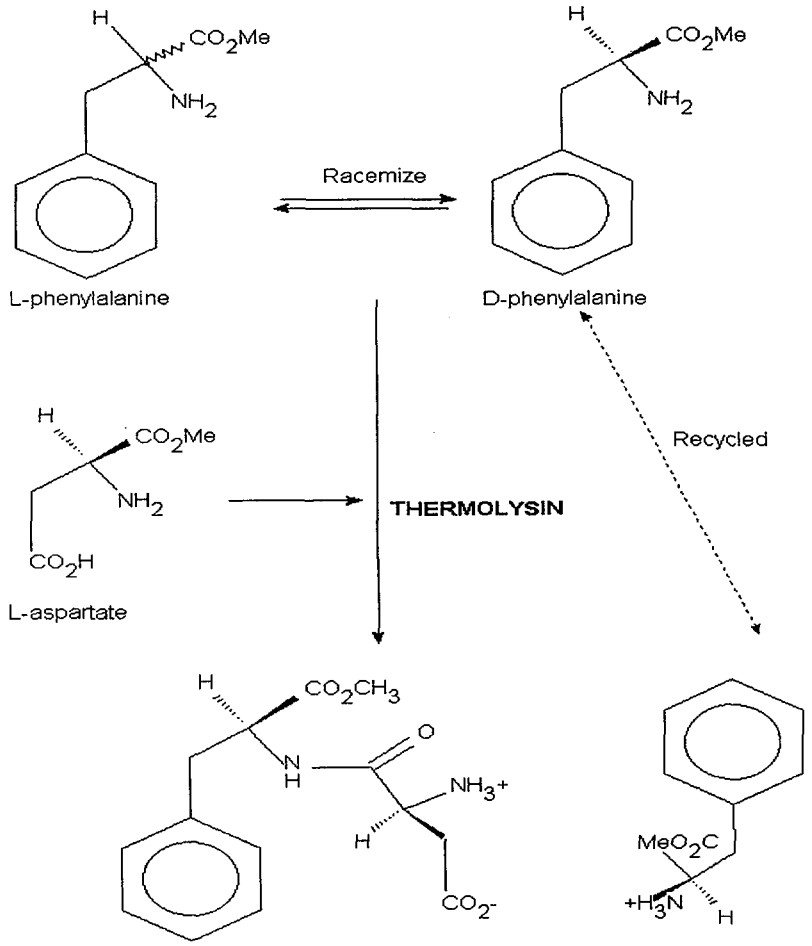


Figure 4. The manufacture of aspartame [14]



Conclusions

The industrial use of enzymes has rapidly developed during the past decade and is steadily increasing due to their low environmental impact. Advances in recombinant DNA techniques, enzyme engineering, and the screening of novel enzymes will continue to improve the enzyme properties, thus opening new routes for synthetic processes. The creation of enantioselective enzymes results in enzymes with biotechnologically usable properties that may also allow new applications. In this way, the range of enzyme catalyzed reactions that may be carried out on industrial scale is continuously expanding. New developments in basic research for improving the fundamental knowledge on enzyme behaviour and, as a result, the new biotechnological applications of enzymes are surely going to increase in the near future.

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INTRODUCTION TO COMBINATORIAL CHEMISTRY AND COMBINATORIAL TECHNOLOGIES

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Abstract

The need for combinatorial technologies

Drug discovery in the past has been traditionally based on the random screening of collections of chemically synthesized compounds or extracts derived from natural sources, such as micro-organisms, bacteria, fungi, plants, of terrestrial or marine origin or by modifications of chemicals with known physiological activities (figure 1).

This approach has resulted in many important drugs, however the ratio of novel to previously discovered compounds has diminished with time. In addition, this process is very time-consuming and expensive. A limiting factor was linked to the restricted number of molecules available or extract samples to be screened, since the success rate in obtaining useful lead candidates depends directly on the number of samples tested. Chemical synthesis of new chemical entities is often a very laborious task, and additional time is required for purification and chemical characterization. The average cost of creating a new molecular entity in a pharmaceutical company is around US$ 7,500/compound [1]. Generation of natural extracts, while very often providing interesting new molecular structures endowed with biological properties, leads to mixtures of different compounds at different concentrations, thus making activity comparisons very difficult. In addition, once activity is found on a specific assay, the extract needs to be fractionated in order to identify the active component. Quite often, the chemical synthesis of natural compounds is extremely difficult, thus making the lead development for a new drug a very complex task. The time and cost needed for the development of new drugs have increased steadily during the past three decades (figure 2).

Estimated costs for introducing a new drug to the market now reach around US$ 200-300 million, and this process takes around 10-12 years after discovery. This increase in time and cost is due mainly to the extensive clinical studies of new chemical entities required by competent regulatory agencies, such as the US Food and Drug Administration, and to a lesser extent to the increased costs associated with research. The time and cost required for clinical and preclinical evaluation of new drugs is not likely to decrease in the near future, and as a consequence, a key issue for pharmaceutical companies to stay in the market has been to increase the number of new drugs in the development pipeline. While the pharmaceutical industry was demanding more rapid and cost-effective approaches to discovery, the advent of new methodologies in molecular biology, biochemistry and genetics, which lead to the identification and production of an ever-increasing number of enzymes, protein and receptors involved in biological processes of pharmacological relevance (and are good candidates for the development of screening assay), complicated this scenario even further. The introduction of combinatorial technologies provided an unlimited source of new compounds that are able to satisfy all these needs (figure 3).

Combinatorial technologies

Combinatorial approaches were originally based on the premise that the probability of finding a molecule in a random screening process is proportional to the number of molecules subjected to the screening process. In its earliest expression, the primary objective of combinatorial chemistry focused on the simultaneous generation of large numbers of molecules and on the simultaneous screening of their activity. Following this approach, the success rate to identify new leads is greatly enhanced, while the time required is considerably reduced.

The development of new processes for the generation of collections of structurally related compounds (libraries) with the introduction of combinatorial approaches has revitalized random screening as a paradigm for drug discovery and has raised enormous excitement about the possibility of finding new and valuable drugs in short times and at reasonable cost (figure 4).

However, the advent of this new field in drug discovery did not obscure the importance of "classical" medicinal chemistry approaches, such as computer-aided rational drug design and Quantitative Structure Activity Relationships (QSAR) for example, but catalyzed their evolution instead to complement and integrate with combinatorial technologies.

The word "combinatorial" appeared in the scientific literature at the beginning of the 1990s, but the generation of the first combinatorial libraries can be dated back to the beginning of the 1980s. The first reports dealt with the simultaneous production of collections of chemically synthesized peptides, produced by solid phase methods on solid supports [2-6]. Peptides were particularly suited for combinatorial synthesis, given the well established synthetic protocols available, the great number of different molecules attainable, and the potential to generate leads of biological and pharmaceutical value (figure 5).

The use of peptide libraries was greatly accelerated by the introduction of biological methods for library preparation by the use of the phage display technology, which provided interesting advantages over the synthetic counterpart [7, 8]. At the same time, the first papers on the generation of oligonucleotide libraries appeared in the literature [9, 10], thus suggesting the possibility of extending the applicability of combinatorial approaches to other classes of synthetic or natural oligomeric compounds, such as carbohydrates. There

Figure 1. The basic sources of molecular diversity and definition of libraries

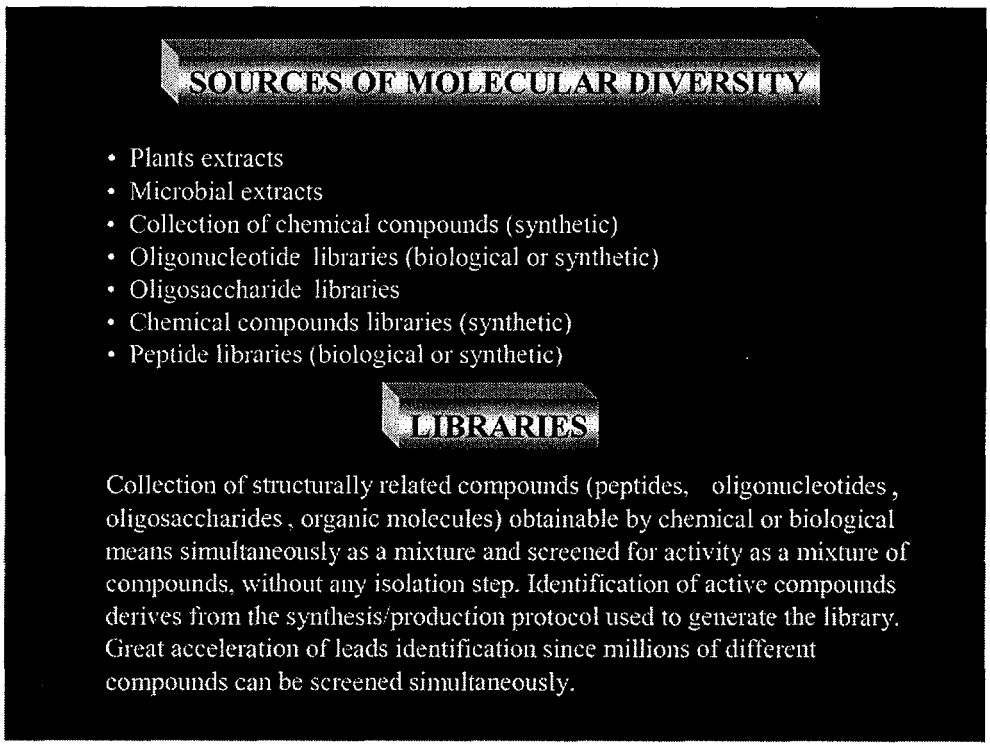


Figure 2. Time needed for new drugs development in the last decades

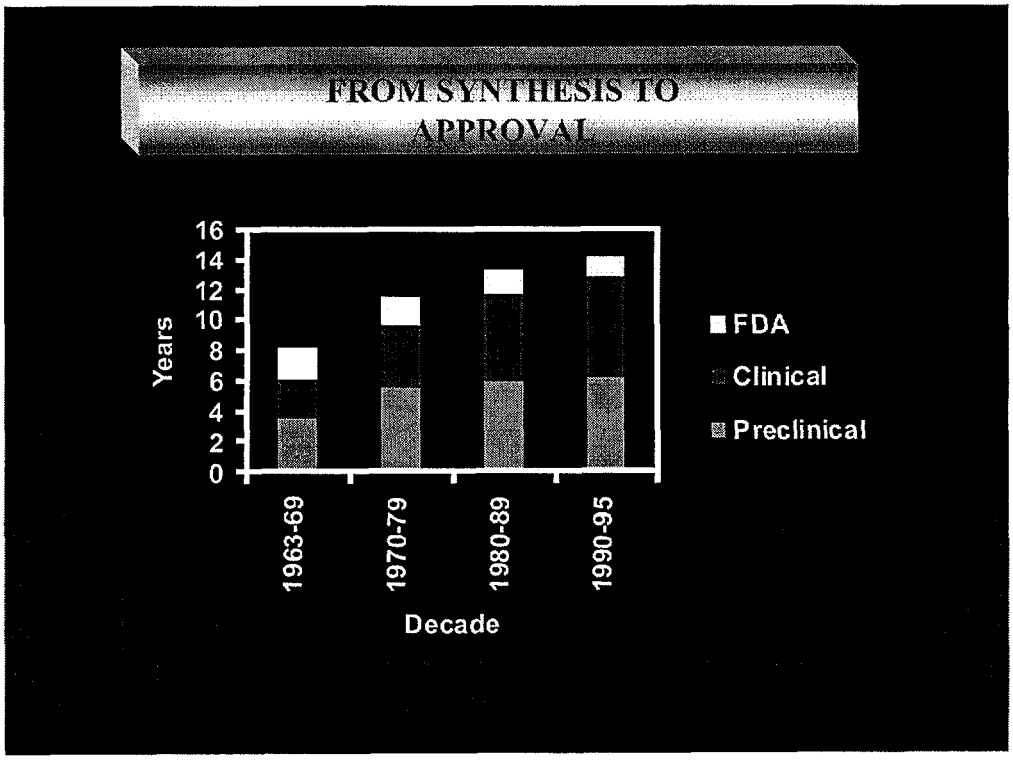


Figure 3. Key factors affecting drug discovery

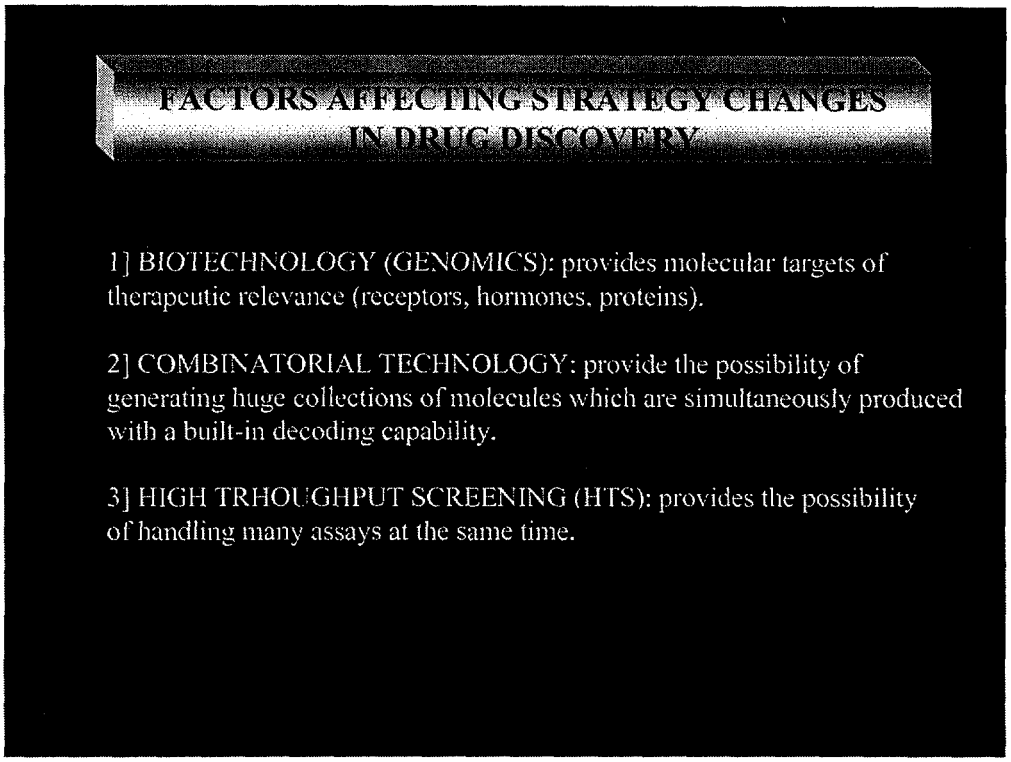


Figure 4. Principal characteristics of conventional vs. combinatorial strategy of drug discovery

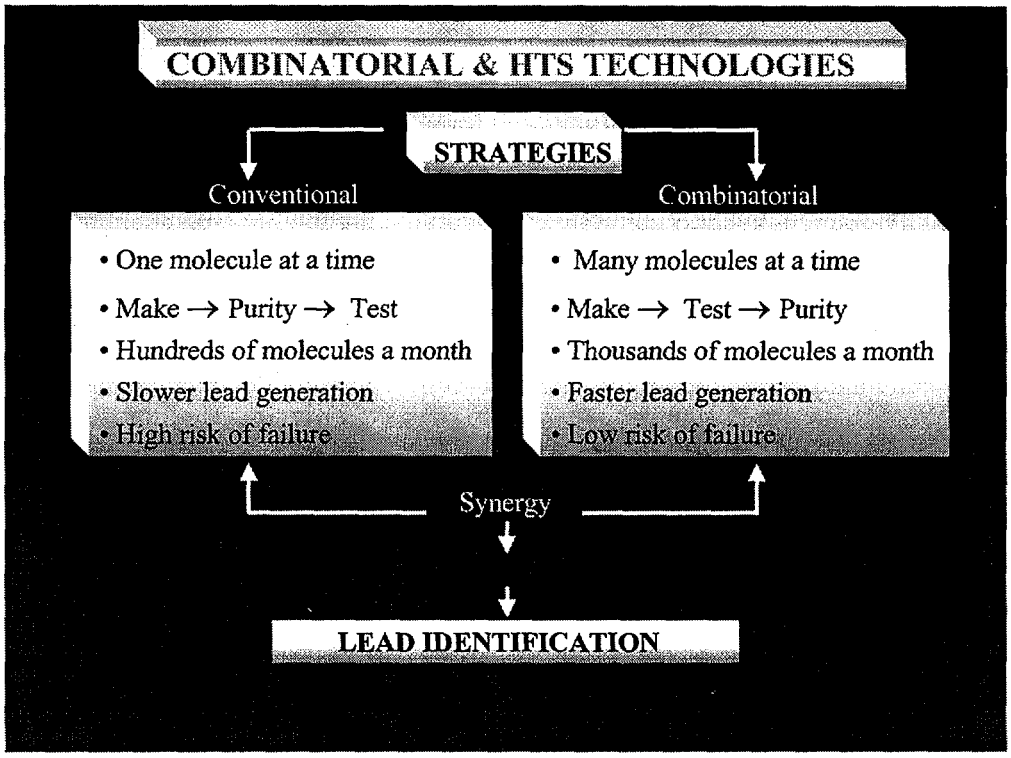
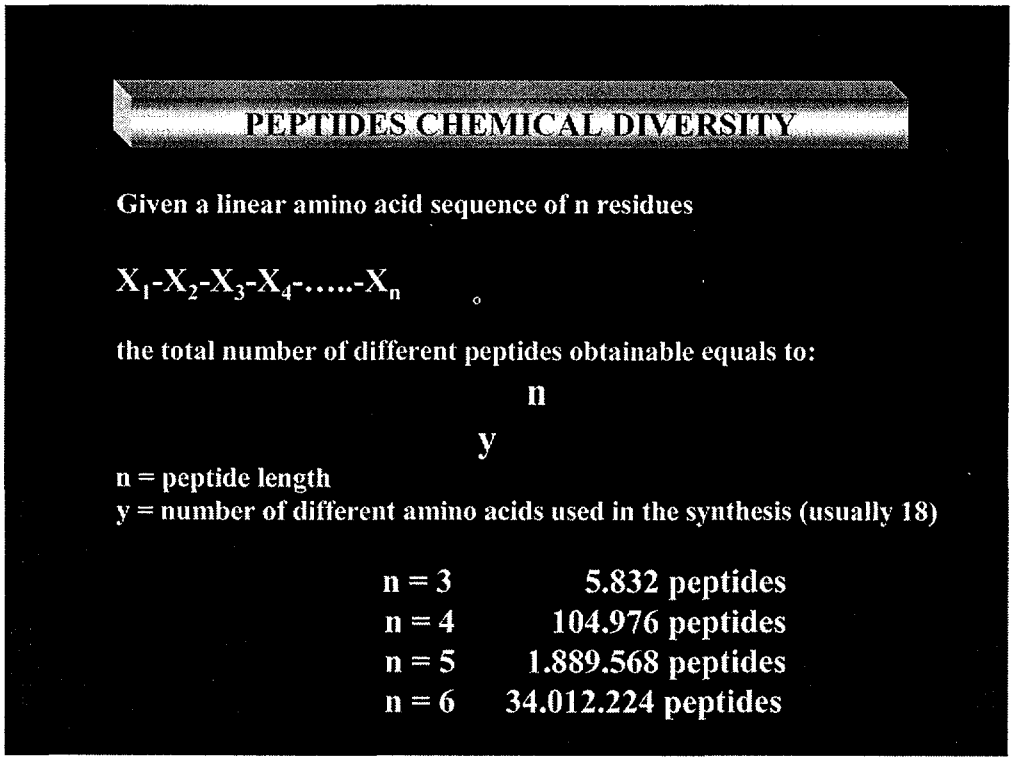


Figure 5. Number of compounds (peptides) generated by combinatorial approach



are many important biologically active glyco-conjugate drugs whose carbohydrate constituents are associated with the molecular mechanism by which these drugs exhibit their effect. With these drugs exploration of carbohydrate molecular diversity has the potential to identify novel agents with enhanced potency (figure 6).

Applicability of combinatorial technologies

Many active compounds have been selected to date following combinatorial methodologies, and a considerable number of those have progressed to clinical trials. However, combinatorial chemistry and related technologies for producing and screening large numbers of molecules also find useful applications in other industrial sectors not necessarily related to the pharmaceutical industry. Emerging fields of application of combinatorial technologies are the diagnostic, down-stream processing, catalysis, and the new material sectors. In the first case, combinatorial chemistry (CC) can be successfully applied to the identification of previously unknown epitopes recognized by antibodies in biological fluids associated to pathological conditions. The selected epitopes can then be used for the development of diagnostic kits useful for the identification and quantification of the antibody of interest. In the down-stream processing field, combinatorial chemistry finds application in the selection of ligands able to specifically recognize macromolecules of biotechnological interest, such as proteins, antibodies or nucleic acids. This is of relevant industrial importance, since the major costs associated with the production of recombinant molecules for therapy is associated to the purification of the desired target molecule from crude feedstocks. The availability of specific and selective ligands to be used in affinity chromatography for the capture and concentration of the target from crude samples will considerably reduce production costs of biopharmaceuticals, such as monoclonal antibodies [11]. Combinatorial technologies have also been applied to the identification of new macromolecules endowed with catalytic activity for reactions where natural enzymes are inactive. This application, even if still at an early stage, is drawing considerable attention from the industrial sector, since the availability of new enzymes may reduce the production costs of many chemicals.

Combinatorial tools

A broad variety of new synthesis and screening methods are currently grouped under the term combinatorial. These methods include parallel chemical synthesis and testing of multiple individual compounds or compound mixtures in solution, synthesis, and testing of compounds on solid supports, biochemical or organism-based synthesis of biological oligomers coupled to selection and amplification strategies. Fully automated instruments for the synthesis and screening of libraries of compounds are integrated tools in combinatorial technologies, as well as computer-assisted approaches for library design. A very important class of molecular libraries is represented by peptide libraries. Peptides are particularly suitable for the construction of libraries, since a high degree of structural diversification can be easily achieved by simply varying the peptide sequence length or by the introduction of different amino acids other than those occurring naturally. The number of different peptides obtainable by a combinatorial approach is governed by the simple formula:

$$N = b^x$$

where N is the total number of molecules obtainable, b is the number of residues used in the construction of the library and x is the sequence length. Generation of synthetic peptide libraries generally follow the divide:couple:recombine process

(DCR) [2, 5], where different aliquots of resin for solid phase synthesis are treated separately with solutions containing different activated amino acids which, after coupling, are recombined, mixed, and then divided again in different aliquots (figure 7). The process is then repeated several times until the desired length of the library is accomplished. After resin cleavage and deprotection, peptides can be tested directly in biological assays. Alternatively, by using resins where the peptide is not cleaved after deprotection, peptide libraries can be tested while still attached to the resin beads by using target molecules tagged with appropriate labels for detection [12, 13]. Several procedures have been reported for the multiple synthesis of peptides [14, 15] or peptide libraries [16], but the majority require the availability of automated instruments or tailored laboratory equipment, which is not always available in most laboratories. However, simple procedures for the multiple synthesis of peptides or for the preparation of peptide libraries in the micromole scale have been developed, requiring only very common laboratory equipment, such as a vortex equipped with a sample holder for 25 eppendorf tubes and a small centrifuge for polypropylene test tubes [17]. In addition to peptide libraries synthesis, this procedure can also be very conveniently applied to the simultaneous small-scale manual synthesis of at least 30 different peptides.

Synthesis starts suspending 0.1 mmol of resin for solid phase synthesis in 9 ml of a DCM:NMP (6:4 v/v) mixture. This solvent composition permits a homogeneous dispersion of the resin, making the aliquoting for libraries preparation very simple and convenient by simply pipetting the desired amount of suspended resin in the polypropylene test tubes. Subsequently 1 ml of NMP is added to each aliquot which, after vortexing, is centrifuged. In this solvent mixture (DCM:NMP 1:4 v/v) resin separation from solvent proceeds very easily. Excess solvent is then removed by vacuum aspiration, using a needle connected to a water vacuum pump. Each different resin aliquot is then treated with a solution containing the appropriate activated amino acid. This method provides a low-cost and easy approach to peptide libraries synthesis for laboratories whose synthesis requirements do not justify investment in an automated peptide synthesizer. All the reagents and laboratory instruments are commercially available at very low cost, and there is no need for custom-made apparatus. In addition, the method has a more general applicability, since it can be used whenever a solid phase synthesis has to be performed, either to prepare libraries (linear, multimeric, cyclic, or peptoidic) or single peptides. The small synthesis scale employed allows the preparation of only few micromoles of peptides, but this amount is more than adequate for a vast array of biological assays.

Figure 6. Diversity of compounds generated by combinatorial approach

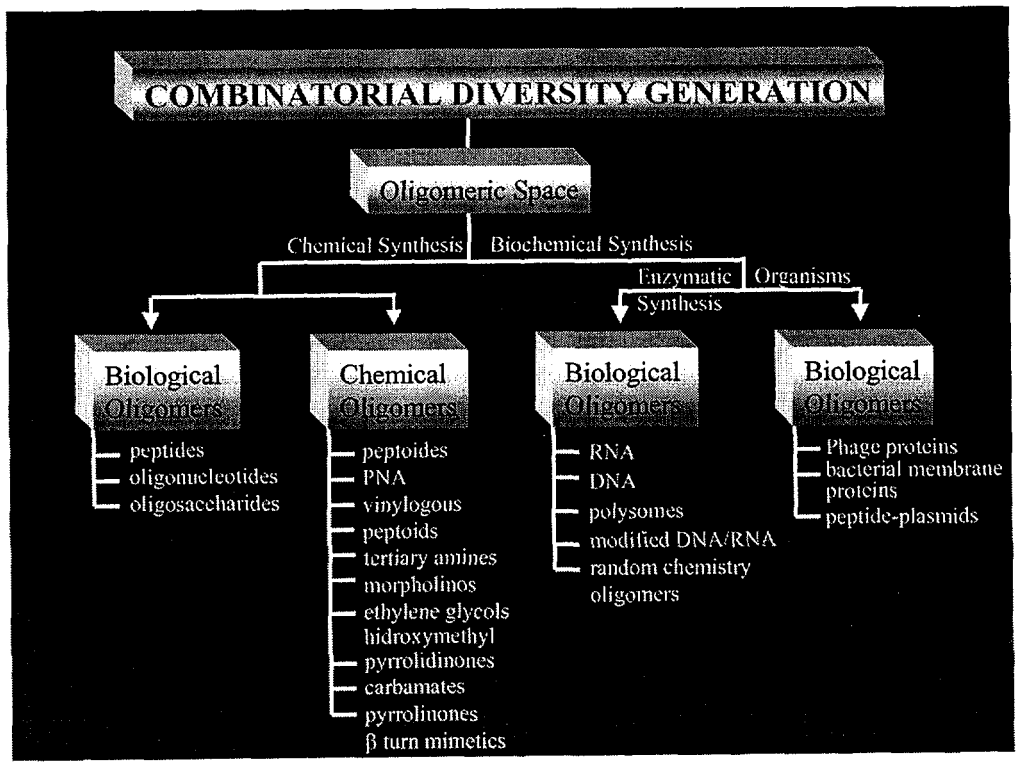
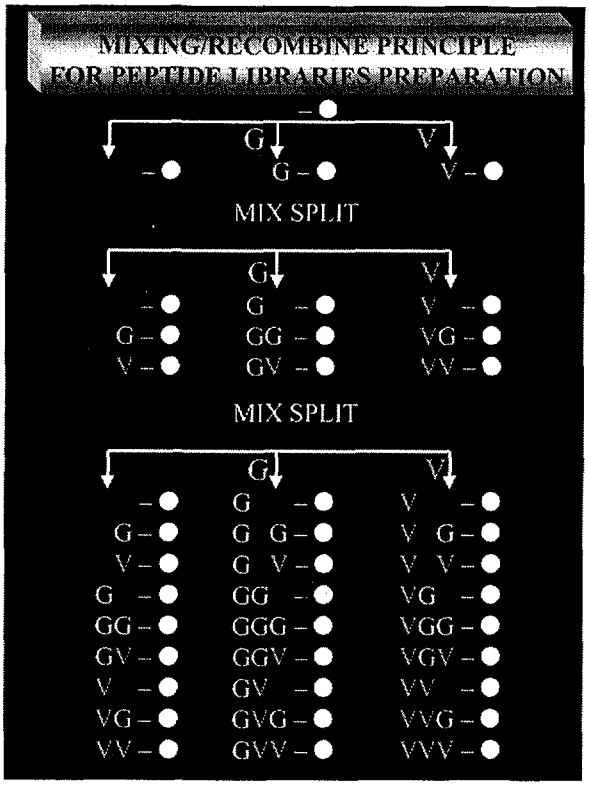


Figure 7. The principle of "Mix-split" combinatorial approach



Peptide libraries could even be manually synthesized in laboratories where combinatorial chemistry could be introduced without investments.

Another important aspect of combinatorial chemistry is the analytical characterization of molecular libraries. Since a considerable number of different molecules are tested separately or in combination, analytical data should indicate that all the expected components occur with a comparable degree of purity. Amino acid analysis by TOF-MALDI mass spectrometry for peptide libraries quality control is often used. Amino acid analysis is useful mainly for the characterization of amino acid-based libraries (peptides, benzodiazepines, hydantoins). It is not sufficient to only give conclusive information on the actual composition of a library, but it represents a rapid and versatile approach to evaluate the distribution of components in that mixture or check the presence or absence of a given peptide family. This method has the great advantage that it can be applied with both soluble (lyophilized) or support-bound libraries, since the conditions of hydrolysis are strong enough to remove the peptides from any kind of resin or other solid surface, such as paper, cotton or glass. The main drawback with the use of data obtained from amino acid analysis when judging the quality of a given library is that side products deriving from incomplete side chain deprotection or side chain modifications cannot be detected, since the integrity of the amino acids during hydrolysis is often restored. The presence of side products is best investigated using mass spectrometry methods (tandem mass spectrometry). Mass spectrometry methods are powerful tools for the analysis of mixtures of compounds from any source. Different techniques such as Electrospray (ES), Matrix Assisted Laser Desorption Ionization (MALDI), Fast Atom Bombardment (FAB) and tandem mass spectrometry have been successfully used to evaluate the composition and purity of synthetic peptide mixtures, but there are no limitations for their use with purely organic libraries. When interfaced to High Performance Liquid Chromatography (HPLC) or capillary electrophoresis, the ES becomes the most powerful method for the characterization of even very complex mixtures, since the combination of the two techniques allows the identification of compounds with very similar chemical properties. MALDI is a very a sensitive method and can be used when very small amounts of sample are available.

In combinatorial chemistry, due to the high number of chemical manipulations required to synthesize libraries of compounds and the high number of screening steps, automation is unavoidable (figure 8). Many research groups, both in academia and industrial settings are developing automated instruments specifically tailored to these needs, and this field of technology is acquiring an extremely important role in the development of combinatorial technologies for the next millennium. However, semi-automated instruments requiring little investment may be constructed in research labs operating on a low budget.

The screening steps required to decipher the active sequence from a molecular library are strictly related to the type of library used, to the synthesis or preparation cycles needed, and to the kind of activity wanted. Molecular libraries can be prepared following chemical or biological approaches. For the first case, libraries can be prepared free in solution or anchored to solid supports, requiring two different screening procedures. Resin-released libraries can be conveniently used in the search for molecules able to interfere in solution with a specific biochemical recognition event, such as in the case of hormone-receptor, antigen-antibody, or inhibitor-enzyme

interactions. Screening can be conveniently performed in evaluating the inhibitory activity of sub-libraries, where the nature of at least one functional group of the library is known in a predetermined position on the assay under consideration (figure 9).

This allows the identification of the first functional group in the library responsible for the activity. The complete deciphering of the active structure must then follow iterative cycles of synthesis and screening steps, where other sub-libraries are prepared, all of them with the previously identified functional group in the predetermined position (n) on the scaffold, and for all of them with the functional groups in the n+1 position known. The sub-libraries are screened again for activity, leading to the identification of the n+1 functional group responsible for activity. The number of iterative synthesis-screening cycles consequently depends on the number of different functional groups in the library. Alternatively, soluble libraries can be immobilized, again in the sub-library format, on solid supports such as microtiter plates for Enzyme-Linked Immunosorbent Assay (ELISA) determination. The target molecule, labelled with a reporter compound such as chromophores, radioactive isotopes, biotin, or enzymes, is incubated on the plates. The sub-library with the highest activity for the target will be easily detected, and repeating, as before, iterative cycles of synthesis and screening the structure of the active compound.

In combinatorial chemistry many different types of libraries can be produced by using solid phase or solution phase methods (figure 10).

The different technologies and strategies used in the production of combinatorial libraries are now so well developed that it is easy to plan synthetic schemes for the generation of a huge number of compounds. Since the rate at which compounds can be screened constitutes a limit to the use of combinatorial technologies, it is important to be selective about the compounds which are synthesized (figure 11).

Computational methods are very valuable from this point of view to assist in the design of combinatorial libraries. The main requirement for lead generation is often to maximize the range of structural types within the library with the expectation that a broad range of activities will result. As a consequence, diversity analysis is an important aspect of library design. The diversity of libraries may be measured by the use of similarity or dissimilarity indexes which make intermolecular comparisons possible. Measures of chemical similarity have been developed for similarity searching in chemical databases. The calculation of the similarity between two molecules involves the characterization of the molecules by using chemical/structural descriptors, followed by the application of similarity coefficients to quantify the similarity.

Biological methods for library preparation are mainly limited to peptide or oligonucleotide libraries. For peptide libraries, methods are based on the construction of a pool of clones, each one expressing a different peptide on its surface (figure 12).

The peptides are fused to proteins normally expressed on the surface of the micro-organism used. Phage display libraries are the most commonly used. Screening is accomplished by incubation of the target molecule, adsorbed to a solid support with the phage population. Active phages will bind the target even after extensive washing steps. Target-bound phages are isolated and propagated by infection of *E. coli* and subjected to an additional round of adsorption to the immobilized target. This procedure increases both the number of active phages and the stringency of selection, since harsher conditions may be employed in the washing steps to reduce the number of non-specifically bound phages. As in the case of synthetic libraries, iterative cycles of

Figure 8. Role of automation in CC/CT

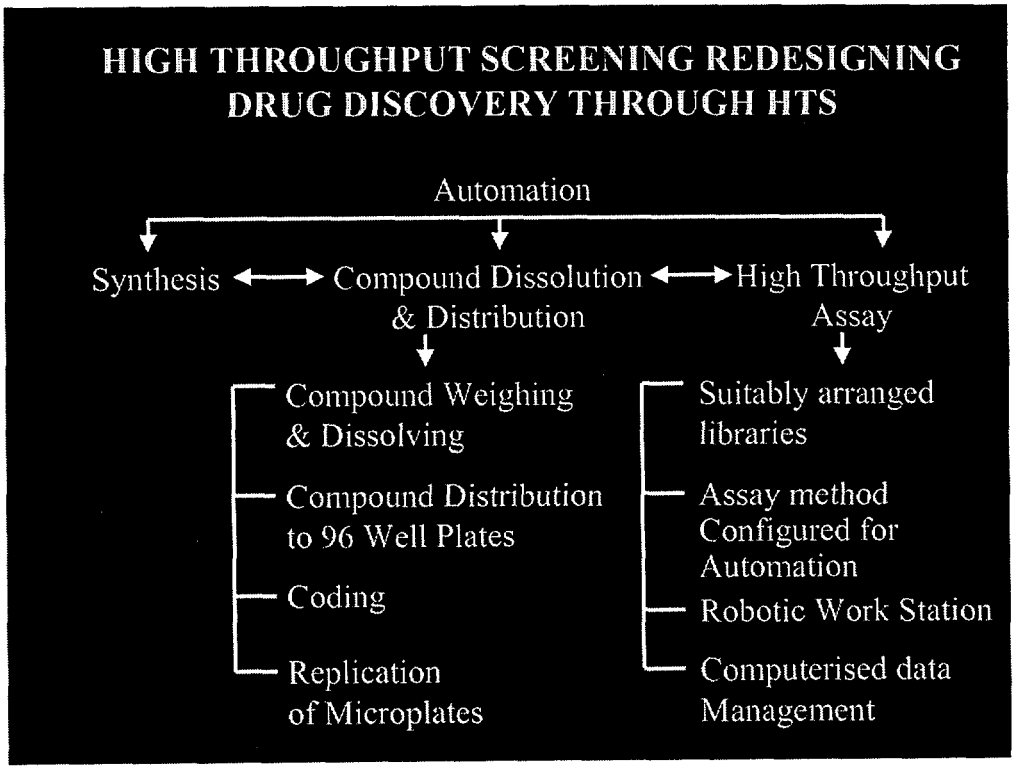


Figure 9. Conventional use of screening sub-libraries

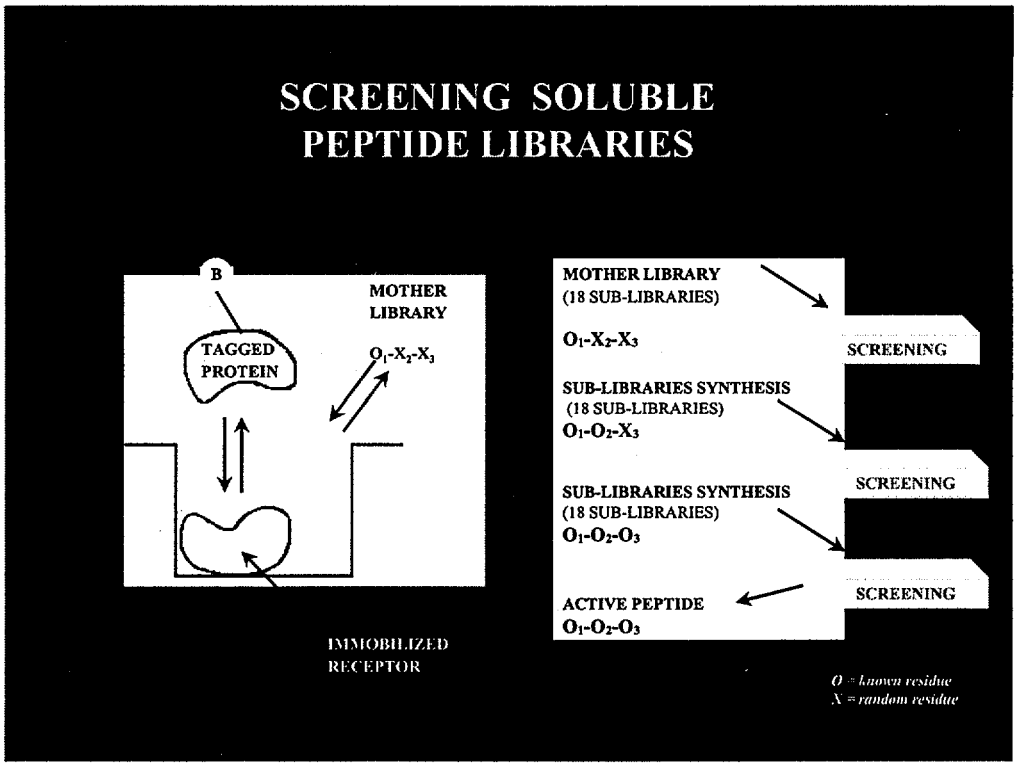


Figure 10. Characteristics of solid phase and solution phase combinatorial chemistry

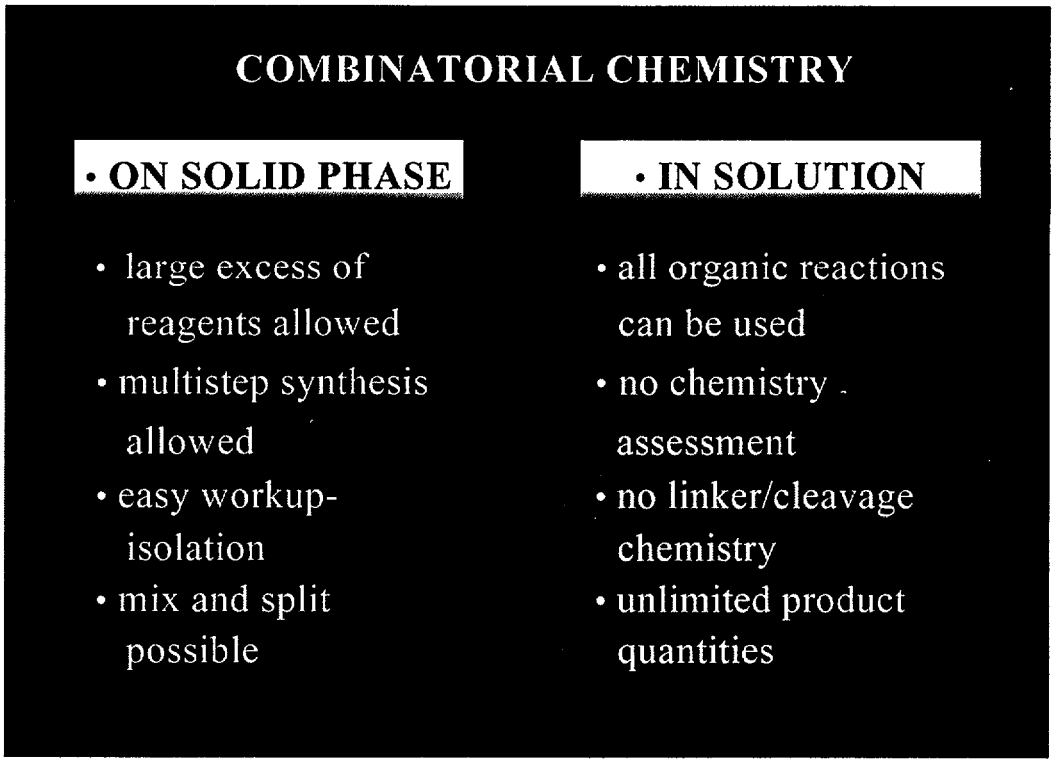


Figure 11. Chemical diversity and number of molecules produced by various concepts of synthesis

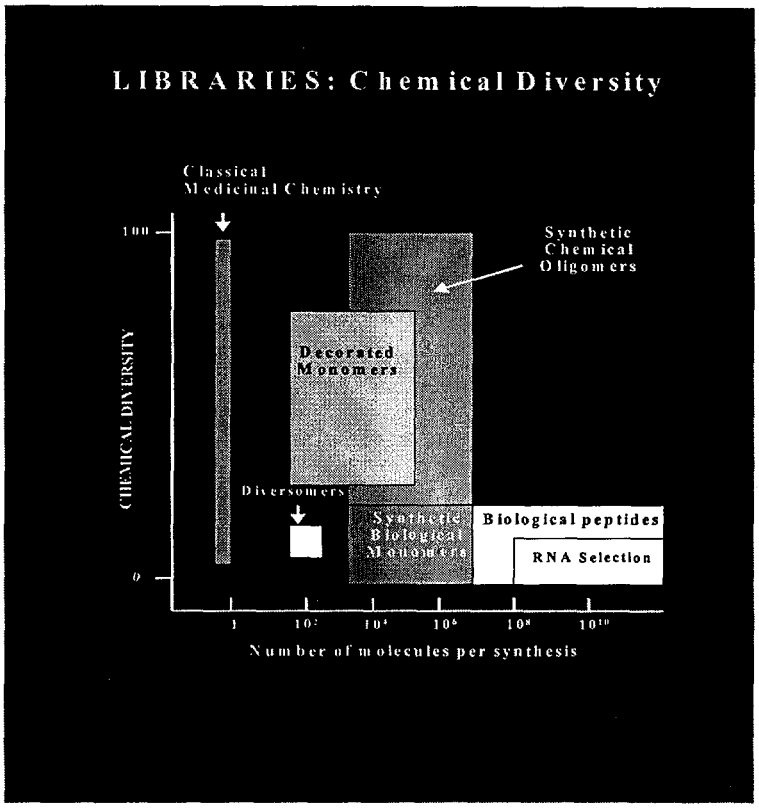
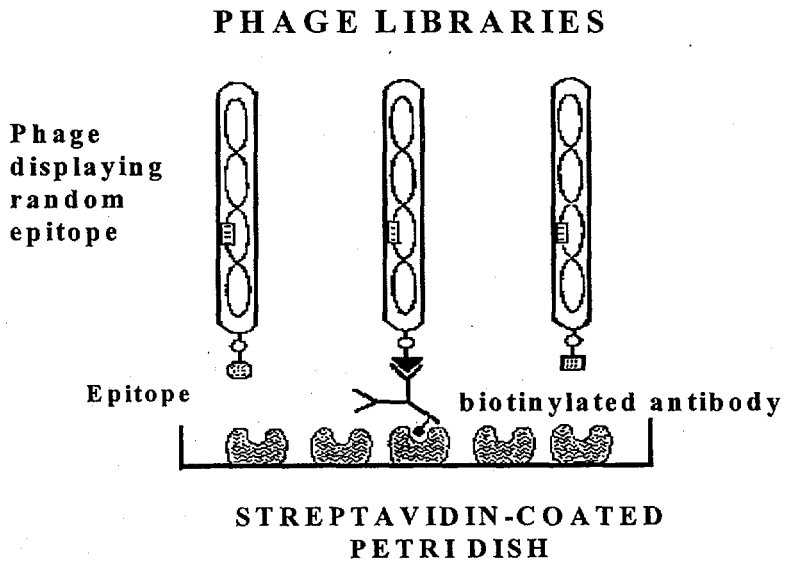


Figure 12. Principle of phage libraries preparation



adsorption, washing, elution and propagation in *E. coli* are performed to enrich the phage population in the active or in few active sequences. Active phages may then be subjected to DNA sequencing in order to decode the active peptide sequence.

The use of biological display libraries for the isolation of peptide ligands is an interesting alternative to chemical libraries. Since 1985 [18], when this technique was first published, many fields of research have benefited from its use. Biological display libraries are constituted by large pools of micro-organisms (up to 10^9-10^{10}), each one expressing a different polypeptide on its surface. These libraries can be easily propagated and used in repeated cycles of selection over the target molecule. In a typical experiment, the library is incubated with the target bound to a solid support; the bound micro-organisms are eluted, grown and selected over the target 2 to 4 more times. At the end, single clones are easily isolated and analysed.

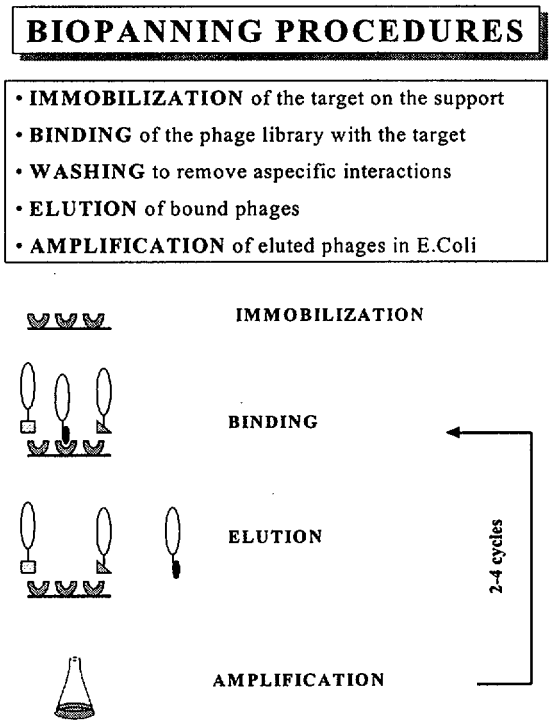
The construction of biological display libraries requires the introduction into a micro-organism of the genetic information necessary for peptide synthesis. For the construction of a random peptide display library it is necessary to synthesize pools of DNA fragments that are then inserted into specific vectors. The DNA fragments are chemically syn-

thesized as a mixture of single-stranded degenerated oligonucleotides containing constant regions and one or more degenerated stretches of DNA. DNA consists of sequences of four different nucleotides, each trinucleotide coding for a corresponding amino acid. Because of the codon degeneracy, most of the amino acids are coded by more than one triplet. Since in fully degenerated oligonucleotides there is the possibility of introducing stop codons that will interrupt protein synthesis, the oligonucleotides are synthesized using different mixtures of nucleotides, especially in the third position of each triplet [19]. The DNA fragments to be cloned must be in a double-stranded form, at least at the end of each fragment. This is normally done by annealing short oligonucleotides to a complementary constant region inserted during synthesis and by enzymatically completing the complementary DNA strand. After compatible ends are prepared by restriction enzyme digestion, the fragments are ligated into an appropriate vector and then introduced into the microorganism.

The most common micro-organism used for peptide display is the *E. coli* filamentous bacteriophage [19]. Bacteriophages are viruses that infect bacteria by injecting their single-stranded DNA genome into the bacterial cells. Once inside the cell, they start to replicate their DNA. By using the host protein synthesis machinery, their coat proteins are synthesized and the DNA packaged into phage particles across the bacterial membrane and secreted into the medium from which they are easily recovered by precipitation.

The ligand selection process from biological libraries is called biopanning (figure 13). The target molecule must be bound to a solid support, usually a microtiter plate or a small

Figure 13. Principles of biopanning



Petri dish. Less common alternative supports are magnetic particles, column with solid matrices, cells, or mammalian organs. In a typical experiment, the number of phages that are incubated with the target corresponds to about 100 to 1,000 times the complexity of the library. After the unbound clones are washed away, the bound ones are eluted by different methods, like low pH, high concentration of free target, direct infection of bacteria cells. The eluted phages are grown, purified and submitted to a new cycle of selection. Usually 3 to 4 rounds of selection are sufficient, and the entire process can be completed in about a week. At the end, several clones are isolated and their DNA extracted and sequenced. The DNA portions coding for the peptides are translated into amino acids and the sequences compared. If a consensus sequence can be identified, the screening may have been successful. One or more peptides are chosen and chemically synthesized in order to verify their binding affinity, outside the micro-organism system.

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ICS-UNIDO programme on catalysis and sustainable chemistry

Different activities are being organized and developed by ICS-UNIDO (International Centre for Science and High Technology of the United Nations Industrial Development Organization headquartered in Trieste) to promote and transfer knowledge and technologies on catalytic systems and their industrial application. It was clearly stated by the Expert Group Meeting of ICS-UNIDO held in Trieste (27-29 April 1998) on "Trends in Catalysis for Industrial Applications", that catalysis is the key to efficiency of chemical reactions offering new technologies for use in the production of fine chemicals, drugs, agro-chemicals, special chemicals, and so on. Its application can allow the development of environmentally sound chemical processes, key elements for promoting industry geared towards pollution prevention. ICS is evaluating and promoting cooperation projects with developing countries, focused on new, cleaner processes and the optimization of those already used in industrial production. Efforts supported by ICS are channelled into improving catalyst performance in fine-chemical production lines, in particular of the SMEs. There are immediate impacts on quality and quantity of by-products or waste generated.

Another ICS-UNIDO action is the organizing of workshops, expert group meetings and training courses on these topics. Recently, the Workshop on "Catalysis in Fine Chemistry" was held in Rio de Janeiro, Brazil, on 17-21 May 1999. Two other activities are realized in this period: an Expert Group Meeting focused on "Guidelines on Chemical Technologies based on Catalysis" in Trieste, Italy (14-15 June 1999) and a Workshop on "New Catalytic Systems and Processes Applicable to SMEs: The Role of Modelling Process Simulation" in Pune, India (8-12 November 1999). The activities foreseen for the years 2000-2001 are concentrated on catalysis and pollution prevention as an important tool for sustainable development.

B. NEWS AND EVENTS

United Nations and other organizations' news

Indian science chiefs

Two major Indian research centres have new leaders to take them into the next century. The International Centre for Genetic Engineering and Biotechnology (ICGEB) in New Delhi, devoted to fostering biotechnology in the developing world, has a new director, malaria researcher Virander Singh Chauhan, as of 15 July 1998.

Observers hope this means a turnaround for ICGEB. Directorless for almost a year, the institute "suffered badly" from neglect in recent times, failing to cultivate industry collaborations, for example. Chauhan says he hopes to spur modernization of ICGEB, which relies on dues from 52 member nations.

Also getting a new leader is India's premier research centre, the Indian Institute of Science in Bangalore. On 1 August 1998 chemist Goverdhan Mehta, vice chancellor of the University of Hyderabad, will take over. This marks the first time in 30 years that an outsider has the leadership job at the institute, whose $25 million annual budget supports 475 scientists. (Source: *Science*, Vol. 281, 17 July 1998)

It's life, as far as we know it

A complete list of the world's known 1.5 million species could be available on the World Wide Web—along with links to relevant scientific papers—by early next century. The major beneficiaries should be poor nations that face severe problems conserving their rich biodiversity because they have few resources to catalogue it.

The $300-million project, dubbed the most ambitious ever in biodiversity, will link together information stored in museums and research institutes around the world. A description of each species will be hyperlinked to references in scientific papers and information on where the organism is found.

This catalogue of life is known as the Global Biological Information Facility (GBIF) and is the brainchild of a working group of the OECD's Megascience Forum, which discusses the coordination of science projects too large for any one member nation to handle. Details were revealed at an international conference on biological informatics held in Canberra.

Its proposers argue that the GBIF is essential to conserving the world's biodiversity. Rich nations, which hold most biological information and specimens but are home to relatively few species, can help conservation in poorer nations where most species are found.

"Most countries do not even know what biodiversity they have, so they can't mobilize their scientific resources effectively to conserve biodiversity", says Jim Edwards of the US National Science Foundation, who chaired the OECD forum's working group.

But it's not all gain—even the project's supporters are worried about some potential uses for the data. (Source: *New Scientist*, 18 July 1998)

Two Congresses in Mexico in 1999

The Mexican Society of Biotechnology and Bioengineering and The Latin American Association of Biotechnology and Bioengineering announce the VIII Mexican Congress of Biotechnology and Bioengineering and IV Latin American Congress of Biotechnology and Bioengineering which will be held simultaneously at Bahías de Huatulco, Oaxaca, México, from 11 to 17 September 1999, to analyse and discuss advances and perspectives in biotechnology and bioengineering.

Persons from industry and academia interested in submitting papers for presentation at the Congresses, should send a one page abstract by 7 May 1999. In the second announcement, available in February 1999, detailed instructions for paper presentations and information of congress fees, invited speakers, etc., will be provided.

Basic aspects, industrial applications and policy issues in all fields of biotechnology will be included in the Congresses.

Likewise, companies, institutions and suppliers of goods and services interested in participating in the Congresses, can do so as sponsors and/or exhibitors. There will be a very limited number of exhibition booths where companies will be able to exhibit products and/or services and distribute information.

For more information, please visit the Mexican Society of Biotechnology and Bioengineering web site at: http://www.smbb.org.mx or contact:

Dra. Mayra de la Torre, Chairman of the Organizing Committee, Depto. de Biotecnología, CINVESTAV-IPN, México, D.F. México. Tel: (52) (5) 747-70-84 or 747-70-00,

ext. 3900, 3903; Fax: (52) (5) 747-70-02 or 747-70-00, ext. 3905. http://servidor biotech.cinvestav.mx

Dr. Octavio T. Ramírez, Chairman of the Scientific Committee, Instituto de Biotecnología, UNAM, Cuernavaca, Morelos, México. Tel: (52) (73) 29-16-46 or (52) (5) 622-76-46; Fax: (52) (73) 17-23-88; e-mail: huatulco@ibt.unam.mx

Guidelines on HIV/AIDS and human rights

The HIV/AIDS epidemic continues to spread throughout the world at an alarming rate, bringing in its wake widespread abuse of human rights and fundamental freedoms. Many people with HIV/AIDS suffer discrimination, intolerance and prejudice. It is against this background and during the 50th anniversary year of the Universal Declaration of Human Rights, that the Office of the United Nations High Commissioner for Human Rights and the Joint United Nations Programme on HIV/AIDS (UNAIDS) have published the *International Guidelines on HIV/AIDS and Human Rights*. The guidelines, which offer concrete measures to protect human rights and health where HIV/AIDS is concerned, are important not just for people living with HIV but for society in general.

Creating an environment in which there is respect for the human rights of people living with the virus or affected by it in other ways (for example AIDS orphans) will help them live with dignity and without discrimination, according to UNAIDS. Such an environment can also reduce the numbers of people vulnerable to infection. Strengthening the human rights of women, children and marginalized groups is an important first step. Because the human rights of such groups have been eroded in a number of countries, they are disproportionately affected and have more limited access to resources to prevent or treat infection. In a climate of discrimination, people are less likely to present themselves for voluntary HIV testing and are thereby denied treatment, care and support. This also hinders efforts by public health authorities to control the epidemic.

The measures in the guidelines follow three broad approaches:

- Improving governments' capacity to take on responsibility for dealing with the issues, encouraging them to coordinate action across ministries, NGOs and communities, and to promote a supportive environment for groups vulnerable to HIV/AIDS;
- reforming laws and legal support services and focusing on anti-discrimination, protection of public health and the improvement of the status of women, children and marginalized groups; and
- increasing private sector and community participation in the response to HIV/AIDS, including building the capacity and responsibility of civil society to respond ethically and effectively.

The guidelines, which call on governments and communities to confront the issues with a sense of urgency, stress that fulfilment of a state's obligations concerning rights to non-discrimination, health, information, education, employment, social welfare and public participation is crucial to ensure human care and support for those infected and affected by HIV/AIDS. The guidelines also highlight the fact that in the context of UNAIDS, human rights and public health are inextricably linked.

For more information: UNAIDS, Ann Winter, Communications and Public Information, 10 avenue Appia, 1211 Geneva 27, Switzerland. Tel: +(41 22) 791 4577; Fax: +(41 22) 791 4188; e-mail: wintera@unaids.org (Source: *Go Between n° 68*, February-April 1998)

The WHO gets tough on TB after criticism from scientists

Large parts of the developing world are failing to halt the spread of tuberculosis, say the WHO. A report on the 22 countries with the highest incidence of the disease says that in 16, including South Africa, Russia and Mexico, progress has stalled. In some of these countries, including Nigeria and Ethiopia, the situation is rapidly getting worse.

The WHO suggests that the number of new cases worldwide will rise from the current 7 million a year to 10 million by 2015.

Acknowledging that there is a global TB crisis, WHO officials are now pinning the blame squarely on the shoulders of individual governments. They are especially critical of countries such as Russia, Indonesia and South Africa, which have enough money to beat TB but are not taking the threat as seriously as they might. "I call on the governments of these countries to give TB top priority", says Arata Kochi, director of the WHO's global TB programme.

Kochi and his colleagues want the countries singled out by the WHO to follow those, such as Peru and Viet Nam, that have embraced a strategy called directly observed therapy, short course (DOTS). This involves giving patients a combination of drugs for six months under supervision.

The richer developing nations, such as Brazil and South Africa, might be shamed into adopting DOTS by the WHO's newly aggressive stance. But poorer nations in Africa—where the disease's incidence is increasing rapidly—will probably remain in the grip of TB.

Hopes for new approaches rose as the British drugs company Glaxo Wellcome announced plans to spend an extra £10 million on TB research. (Extracted from *New Scientist*, 28 March 1998)

Best-laid plans

The WHO's plans to eliminate seven major diseases may cost much more than the $7.5 billion the agency has budgeted, and the goal could even harm public health by soaking up money from other programmes, claim some US officials.

The diseases targeted for eradication or "elimination"—reducing them to such a low incidence that they are no longer a problem—are lymphatic filariasis, guinea worm disease, polio, leprosy, measles, river blindness and Chagas' disease.

The polio eradication campaign should be completed by 2000 at a cost of $1.6 billion. Programmes on leprosy and guinea worm disease are also making good progress. But the General Accounting Office (GAO), the investigative arm of the US Congress, has doubts about other WHO plans, such as its goal of eliminating lymphatic filariasis, or elephantiasis, by 2030 at a cost of $228 million.

David Heymann, the WHO's director of communicable diseases surveillance and control, agrees that the estimates may have to be revised, but he says the programmes will save money and lives in the long run. (Source: *New Scientist*, 30 May 1998)

Sterile flies conquer sleeping sickness

Tsetse flies, which spread sleeping sickness, have been eradicated from Zanzibar, an island off Tanzania's east coast, by the release of millions of sterile males. Once free, the insects—which had been irradiated—mated fruitlessly with wild females.

The $5-million tsetse project was spearheaded by the International Atomic Energy Agency (IAEA) in Vienna. Working with Tanzania's Tsetse and Trypanosomiasis Research Institute in Tanga, IAEA scientists produced 70,000 sterile males each week.

The sterile male technique had previously been used to control the medfly, which attacks fruit crops across the Americas, and the New World screw worm, whose larvae eat into the flesh of livestock. Now the plan is to extend the tsetse programme to the African mainland, where 300,000 people are infected with the trypanosome parasites that cause sleeping sickness. The disease also affects cattle, doing some $1.2 billion's worth of damage every year.

Arnold Dyck, the project's manager, who was seconded to Tanga from the IAEA, says Zanzibar was ideal for testing the technique because it is isolated and harboured only one species of tsetse fly. The mainland presents a sterner challenge, says Dyck, since 22 species of the fly infest the 36 countries of sub-Saharan Africa.

The team will start by focusing on isolated regions with just one species of tsetse fly. The first big project will cover 25,000 square kilometres in Ethiopia's Southern Rift Valley. (Source: *New Scientist*, 29 November 1997)

Biowarfare sleuths

Countries violating the international ban on biological weapons could be caught out by a new Internet database that will highlight unusual outbreaks of disease.

The 1975 UN Biological Weapons Convention, which bans all military use of biological agents, has more than 150 signatories. Yet intelligence sources in the US estimate that at least a dozen of these countries have covert biowar programmes.

The convention is very difficult to police, in part because most toxins have peaceful uses. The botulism toxin, for example, is used as a muscle relaxant in some medical treatments.

Disease outbreaks caused by accidental leaks of the micro-organisms used in bioweapons production may provide the best indication of a treaty violation. Epidemiologists can distinguish such mishaps from natural outbreaks if they know enough about the strains involved and the patients' histories. But currently such sleuthing can take years. For example, conclusive evidence that a 1979 anthrax epidemic in Ekaterinburg, Russia, started with a leak from a military factory emerged only this year.

To speed the detective work, Al Zelicoff at Sandia National Laboratories in New Mexico has set up a database on the Net to which doctors will be able to post details of local disease outbreaks. Changes in disease patterns should show up as they happen. Patient surveys included in the doctors' reports will allow epidemiologists to work out the most likely source of an outbreak.

To demonstrate the idea using data on a pathogen not linked to bioweapons, three hospitals in New Mexico and one in the formerly secret Russian military research city Chelyabinsk-70 have arranged to post details on cases of hepatitis C.

Although some rogue states may ban their doctors from taking part, Zelicoff expects the public health benefits will entice clinics in most countries to participate. Most disease outbreaks reported on the database will be unrelated to biological weapons. But these data will help identify disease hot spots as they appear, making outbreaks easier to control.

Zelicoff hopes eventually to hand his database over to an international body such as WHO.

The WHO currently maintains a disease reporting network which connects large clinics, mostly in capital cities, by e-mail and telephone. Zelicoff says his system will have greater reach by extending to doctors working in smaller, regional clinics.

Epidemiologists say that between 2,000 and 10,000 clinics worldwide would need to become involved for the network to be truly effective. (Source: *New Scientist*, 20 June 1998)

Dust to dust

Out of focus

At the Earth Summit in Rio six years ago, the Global Environment Facility was welcomed by developing countries and non-governmental organizations (NGOs) as an innovative means of redistributing wealth to preserve ecological health. It was adopted as the main source of funding for the UN conventions on biological diversity and climate change. Since then, with $1.6 billion donated by 34 countries, it has approved 230 projects throughout Asia, the Pacific, sub-Saharan Africa, Latin America and the Caribbean. Conserving biodiversity is only one of its aims. It is also charged with combating climate change, protecting international waters and phasing out ozone-depleting chemicals.

Many of the complaints about the GEF come from those it is designed to help. Developing countries say that it mainly addresses environmental problems of concern to the West while ignoring the most crucial issues affecting the Third World.

Decertification, for example, affects nearly 30 per cent of the world's land—1 million hectares in Africa and 1.4 million hectares in Asia—and costs countries $42 billion every year. Yet this problem receives only marginal support. It is only eligible for GEF funding if it affects one of the four agreed areas. "It is time the GEF seriously considered the issue of land degradation", says Mostafa Tolba, the former head of the UN Environment Programme.

Of the projects the GEF does fund, those concerning biodiversity have drawn the most criticism. A review compiled by the GEF secretariat in Washington, D.C. says this is because they are over ambitious, exclude local communities and operate in a scientific vacuum. The underlying reasons for the loss of biodiversity are "often poorly understood", the review says.

A second report, written by 25 independent environmental consultants, is equally critical. It concludes that the fund "had not been able to focus on ecosystems of greatest global importance to the extent that would be desirable".

Gareth Porter, an American consultant from Washington, D.C. and the report's lead author, points out that there has been no scientific attempt to prioritize which ecosystems and species should be targeted. Some scientists argue that priority should be given to ecosystems with the greatest diversity of species, such as rainforests and coral reefs. Others think species confined to particular regions, such as rhinos, or especially vulnerable ecosystems such as mountains and coastlines, ought to top the list.

In the absence of any agreed scientific criteria, the GEF secretariat has decided that any site that has been designated by an international organization as a nature conservation area is of potential "global importance". But as Porter points out, this is not a very discriminating technique. Just three of the designations used to determine grant eligibility—World

Heritage Sites, Ramsar wetlands and Biosphere reserves—cover more than 1,000 sites.

In practice, 60 per cent of the funds have been directed towards the 25 countries with the greatest biodiversity in Africa, Asia and Latin America. Porter, however, says that national governments have sometimes directed money to areas of less than global importance within those countries. Grants have also been given to countries with no globally important sites at all, he claims.

As in most UN agencies, decisions about the allocation of GEF funds are complicated by national rivalries. Porter points out that many of the 32 countries on the GEF's ruling council resist any scientific attempt to rank different ecosystems because they fear it will limit their choices.

Pier Vellinga, a Dutch environmental scientist who chairs GEF's Scientific and Technical Advisory Panel, agrees that a more systematic analysis of biodiversity priorities is required. But he urges sympathy for GEF's plight.

One of the most vexed issues is funding. At the first GEF assembly in New Delhi in April, governments pledged $2.75 billion over the next four years. Although this is nearly twice that spent over the past six years, it is dismissed by developing countries and NGOs as "chewing gum". They argue that $125 billion is really needed.

But one GEF failure disappoints environmental groups more than all others. An explicit aim of establishing the fund was to force the organizations involved to take environmental sustainability to heart. In particular, it was hoped that the World Bank would think twice before investing in development projects that damage environments the GEF is supposed to protect.

This has not happened. Between 1993 and 1997, the bank invested $9.4 billion in fossil fuel projects that will accelerate climate change, and less than $300 million on schemes to prevent it. Across the globe, say environmentalists, the World Bank has backed dams, roads and chemical-intensive agricultural projects that threaten to wreck protected ecosystems. (Extracted from *New Scientist*, 6 June 1998)

Montreal Protocol sets phaseout for methyl bromide

Representatives of 163 nations agreed to a phaseout of methyl bromide, a highly toxic pesticide that is also a powerful ozone depleter, at the tenth anniversary of the Montreal Protocol (9-17 September 1997), but non-governmental organizations (NGOs) present at the meeting criticized the phaseout schedule as too slow to protect public health and said only a handful of countries (including China, Kenya, Italy, Spain and Israel) prevented a much more rapid phaseout of the pesticide. The Montreal Protocol is an international treaty to protect the earth's ozone layer.

The final Montreal Protocol agreement states that industrialized nations will completely phase out methyl bromide by 2005, with intermediate cuts of 25 per cent in 1999, 50 per cent in 2001 and 70 per cent in 2003. Developing countries will phase out the pesticide by 2015 with a freeze on use in 2002 and 20 per cent reduction by 2005.

A coalition of 25 NGOs attending the meeting from more than 16 countries called for a ban of most uses of methyl bromide in industrialized countries in 1999 with a complete ban in 2001 and a ban in developing countries by 2006. They said that although a small group of corporations will gain from continuing use of methyl bromide, its negative health and environmental effects will be felt worldwide. NGOs were discouraged that the heavy lobbying by methyl bromide producers succeeded in preventing a much faster ban. Use is growing rapidly in some regions despite international controls, largely due to aggressive marketing in developing countries by methyl bromide manufacturers. (Source: *Global Pesticide Campaigner*, December 1997)

United Nations agencies promote methyl bromide alternatives

In July the Multilateral Fund's Executive Committee approved 19 projects to develop alternatives to methyl bromide. Projects include demonstration of both chemical and non-chemical alternatives for crops such as strawberries, cut flowers and tobacco in countries around the world. The combined budget for the 19 recently approved projects is more than $3.3 million.

Sixty-one methyl bromide projects in 36 countries worth US$ 7.7 million have been approved under the Fund to date. The majority are in the "project preparation" stage; 17 projects are moving forward with field work and demonstrations.

The Fund was established under the Montreal Protocol to support the transition from ozone depleting substances in developing countries. Projects are coordinated by four "implementing agencies"—the World Bank, the United Nations Industrial Development Organization (UNIDO), the United Nations Development Programme and the United Nations Environment Programme—working closely with developing country governments. Of the four agencies, UNIDO has taken the lead in developing methyl bromide alternative projects.

The Pesticide Action Network has been tracking these projects since the Fund began financing methyl bromide alternatives in 1997. PAN and Friends of the Earth (FoE) recently joined forces to ensure that these resources are spent effectively and on less toxic alternatives. In early June, the two groups established an international system of "Regional NGO Contact Groups" to work on Fund projects and monitor their implementation.

While the agencies have all expressed interest in working with NGOs in project development and implementation, none of the field level demonstration projects approved by the Fund to date include NGO participation.

The PAN-FoE Regional NGO Contact Group system will streamline this process for the agencies by linking project officers with interested NGOs working directly with farmers and/or with public education experts. The Regional Contact Groups are located in Mexico, Chile, Senegal and Malaysia. (Source: *Global Pesticide Campaigner*, September 1998)

ISAAA launches new intellectual property/ technology transfer (IT/TT) initiative

As part of its continuing mission to expand the availability of agricultural biotechnology, the International Service for the Acquisition of Agri-Biotech Applications (ISAAA) has hired Mr. Walter Haeussler, JD, as a special consultant on licensing and legal issues related to proprietary science and technology transfer. Walter Haeussler will strengthen ISAAA's ability to perform its core function: brokering and facilitating the transfer of biotechnology to developing countries for the primary benefit of the rural poor and small scale farmers. Through his expert consulting service Walter Haeussler will work through ISAAA with developing countries and organizations assisting them to negotiate through the proliferating complexities of licenses, patents and ownership issues that can hinder the transfer of biotechnology

applications, even when they are donated by the public or private sector. Desiring to benefit from the modern biotechnology applications in agriculture, several developing countries have repeatedly requested ISAAA to assume a comprehensive role in brokering the acquisition of biotechnology, to develop in-country capacity building opportunities, and to provide for resources in the strategic and policy areas as a way of more efficiently addressing the complicated issues that arise in the biotechnology transfer process. "My task will be to continue with ISAAA's pilot projects, which all aim at pragmatically linking public and private sectors together, and working toward harmonizing property laws" explains Mr. Haeussler. "Only regulatory frameworks developed from a pragmatic standpoint will efficiently assist in building the structural capacity necessary to successfully manage this promising new technology" he adds.

Walter Haeussler comes exceptionally well prepared to this task. He holds a Bachelor of Science in Chemistry and a JD, worked as a scientist in polymer research, as a patent examiner with the US Patent and Trademark Office, and for a decade as an attorney with a private law firm. Following that, he became President of the Cornell Research Foundation in 1983 and oversaw the entire Patents and Technology Marketing operation of Cornell University. He manages Cornell's intellectual property portfolio of over 500 active US patents and 400 pending ones, and more than 600 licensing agreements. With this background, Mr. Haeussler possesses a demonstrated expertise in dealing with proprietary science, licensing and intellectual property.

Although the more than 140 nations of the World Trade Organization have taken important steps towards the development of international regulations governing intellectual property and the transfer of biotechnology, every nation has its own patent, copyright, and trademark laws. Hence every agreement will be different as the national, legal and socio-economic aspects are different and need to be respected. But as these legal differences are hammered out through pragmatic transfer agreements, they will eventually produce a more level playing field for everyone. This is one of the important objectives of ISAAA's work. To date, ISAAA brokered a series of licensing agreements between companies and institutions in developing countries for the use of a host of genes in Latin America, Africa and Southeast Asia, including a use agreement with one of the centres of the CGIAR.

The constantly shifting and evolving scene of IP/TT and biotechnology requires experience, personal contacts, and flexible leadership, and Mr. Haeussler brings all of these to the table. "Imposing external paradigms frequently creates more problems than it solves" he notes, "and I share ISAAA's faith in a pragmatic, realistic approach to the promise of biotechnology".

ISAAA is an international non-profit organization whose mission is to contribute to poverty alleviation, by increasing crop productivity and income generation, particularly for resource-poor farmers, and to bring about a safer environment and more sustainable agricultural development.

ISAAA's objectives are the transfer and delivery of appropriate biotechnology applications to developing countries and the building of partnerships between institutions in the South and the private sector in the North, and by strengthening South-South collaboration.

Contact: Dr. Anatole F. Krattiger, Executive Director, ISAAA. Tel.: +1-607-255 1724; Fax: +1-607-255 1215; e-mail: afk3@cornell.edu (Source: *News Release*, 24 July 1998)

Latest news on the International Molecular Biology Network (IMBN)

In a significant recent development, the International Molecular Biology Network (IMBN) for Asia and the Pacific Rim recently announced the selection of its first 200 members who will assist in fulfilling the IMBN objectives and vision. The member, from various countries in the Asia and Pacific Rim regions were selected based on the criteria of excellence in research and/or professional activities in molecular biology, following an extensive assessment and evaluation process involving special committees of IMBN and EMBO (European Molecular Biology Organization).

Prof. Ken-Ichi Arai, one of the primary movers of the IMBN, also announced that the network's plans to set up an International Molecular Biology Laboratory (IMBL), modelled along the lines of EMBL's Heidelberg facilities, may be realized sooner than expected. Preliminary plans envisage two laboratories, one in Tokyo and one elsewhere in Asia, possibly Shanghai, each housing up to 200 researchers and funded to the tune of $50-$100 million annually. Financial support from Japanese government sources have been promised as well as a possible site. The plan still needs endorsement from the IMBN and financial support from governments and will be discussed at the first IMBN conference to be held in late June 1998 in Seoul. (Source: *Australasian Biotechnology*, Vol. 8, No. 4, August 1998)

Cassava Biotechnology Network

Cassava Biotechnology Network (CBN) was founded in 1988 to mobilize biotechnology to alleviate poverty and assist development in tropical countries through the improvement of cassava (*Manihot esculenta*), one of the world's most important food crops. Supported in large part by the Directorate for International Cooperation (Den Haag, The Netherlands) and headquartered at the Centro International de Agricultura Tropical (CIAT; Cali, Colombia), CBN is a voluntary research consultation network of laboratories, researchers, and farmers in about 35 countries.

CBN's remits are to: first, to ensure the participation of small-hold farmers in research planning for cassava and to support biotechology research designed to target objectives prioritized by farmers; second, to stimulate and coordinate the necessary supporting strategic biotechnology research, including genetic transformation, molecular genetics, and micropropagation; and third, to encourage and enable free transfer of information and biotechnology tools for cassava. CBN has also acted to raise awareness of cassava's importance to global development objectives.

Why is cassava important? Under marginal low input conditions where cereals may yield 1 to 2 tonnes of grain, cassava will yield about double that in dry weight in the form of edible starchy roots. Because of its reliable harvest, cassava has become the most important locally produced food in one-third of the world's low-income, food-deficient countries. Around 500 million people consume cassava daily, a number certain to rise with increasing population. Furthermore, cassava produces a low cost, versatile starch with many potential uses in foods, pharmaceuticals, textiles, paper making, and other industrial markets—markets that will grow as standards of living in developing countries improve.

The near- and medium-term future is both exciting and uncertain for CBN and cassava biotechnology. The network's

first project ended in June 1997. CBN's members are now engaged in designing a new regional structure (Africa, Asia, and Latin America) in which farmers, biotechnologists, and cassava researchers will all participate in a technology transfer phase. This regionalization aims to tap additional resources of leadership and initiative, achieve closer integration with farmers, and allow each region to focus on its particular priorities. However, even as it prepares for what should be most significant years, CBN's future may be compromised by severe cuts in virtually all international budgets for agricultural development.

CBN is at a crossroads. On the one hand, recent breakthroughs in cassava biotechnology have opened opportunities to develop these advances in research with cassava farmers. Public sector cassava biotechnology could advance rapidly to application. However, there are few advanced laboratories researching cassava: perhaps five leading laboratories are researching cassava transformation and 10-15 research tissue culture or molecular genetics. Loss of a few experienced personnel would compromise the transfer of expertise to cassava-growing countries. There is an urgent need for a commitment to continuity, to permit the effective transfer of cassava biotechnology innovations and capability to national programmes. With adequate support, both basic research on methods improvement and the application of transgenic approaches to cassava production could be taking place within cassava-growing countries within as little as 5-10 years, following initial field trials beginning in 2-3 years.

The total investment in cassava development so far is difficult to estimate but may be about $2.5-3 million since 1988, compared to tens of millions of dollars for rice and orders of magnitude more for certain crops of agronomic importance to the developed world. CBN contributed directly to these achievements through the investment of about $50,000 (about 10 per cent of its total small grant funding), which enabled the exchange of expertise and plant tissues between the research laboratories involved. CBN scientific conferences further encouraged dialogue. Such activities compensated for the low levels of funding and acted to accelerate progress. With the basic technology in place, transgenic research in cassava has shifted to optimization of the techniques and production of plants expressing transgenes of potential agronomic importance.

A major part of CBN's mission has been to act as a link between the intended recipients and researchers in the advanced laboratories. This enables farmers to have a significant input in setting priorities for cassava improvement and ensures that advances being made in cassava biotechnologies stay focused on addressing real needs.

In addition, CBN has encouraged discussion and information exchange about innovations possible through biotechnology, which may provide potential solutions to farmers' expressed needs, even though most cassava farmers may not be able to identify the biotechnology options. For example, farmers—including those operating under subsistence conditions—have identified a need for better markets and prices for their surplus production. One of the obstacles to these markets is that cassava perishes within 24-48 hours of harvest.

Transgenic techniques could provide cassava roots with postharvest durability, which could improve the crops' marketability and help stabilize prices. Cassava produces starch more efficiently than any other crop. Useful variations in native starch quality—altering the proportion of amylose to amylopectin, for instance, which changes the physicochemical properties of the polymer—could open new market niches at better prices.

A pilot farmer network within CBN/Latin America was organized in March 1998, and comprises groups working with CBN on priority setting and in biotechnology-assisted participatory research projects. Representatives of these groups will meet biennially at CBN scientific meetings to exchange experiences among farmer groups and make recommendations to the network as a whole. In addition to strengthening farmers' visibility in CBN, the pilot network will provide opportunities for communication concerning technical and regulatory issues relating to cassava biotechnology.

There is now an urgent need to commence programmes of technology transfer. At this time the efforts of CBN are the only existing mechanisms for assuring continuity in this effort. Several cassava-growing countries, including Cameroon, Ivory Coast, Zimbabwe, Kenya, Uganda, South Africa, Malawi, Philippines, Viet Nam, Bolivia, Venezuela, Cuba, Ecuador, and others have requested CBN assistance in developing cassava biotechnology capability. National programmes in such countries as Brazil, Indonesia, and Thailand are working with CBN to develop cassava biotechnology projects and seek opportunities for funding.

Through the generosity of several donors plus the CBN small grants programme, 10-12 researchers are being trained in advanced laboratories and at the international research centres. Much more is necessary if biotechnology research is to become operational beyond the few most advanced cassava-growing countries. (Extracted from *Nature Biotechnology*, Vol. 16, May 1998)

Regulatory issues

EU to amend view on GMOs

The EU council of ministers is to amend its Directive 90/220 on the deliberate release of genetically modified organisms (GMOs) into the environment.

The proposal is due to be scrutinized by the European Parliament in October and should be adopted by December. Following recent debate between consumers and industry over the GMO issue, the council is keen to establish a transparent and comprehensive regulatory framework in this area.

A key area is to establish common principles for risk assessment. The council of ministers is also proposing a classification system for experimental releases. Consent given to any product placed on the market will be for seven years. During this period products are to be monitored and the consent could be extended. (Source: *European Chemical News*, 29 June-5 July 1998)

EU court threat over French oilseed ban

Europe's battle over genetically modified crops could be heading for a showdown in the courts after the European Commission initiated proceedings against France for imposing a moratorium on two varieties of oilseed rape.

The Commission says the French decision, which imposes a de facto EU-wide ban on the crops, violates rules agreed by all member states (*C & I*, 1998, 636). It has complained in writing to the French government, giving it two months to respond. If no action is taken the Commission says it will take the government to court.

France said it was unlikely to comply. There were "fundamental problems" with genetically modified crops, a government spokeswoman said.

The controversy centres around two varieties of genetically modified oilseed rape developed by AgrEvo subsidiary Plant Genetic Systems (PGS). Under EU rules, France must grant final approval before the crops can be marketed in other member states. This is because the original applications for marketing in Europe were filed by the previous French government.

France received Commission clearance in June 1997 and was expected to rubber-stamp the decision. But in July of this year it imposed a moratorium on most genetically modified crops, including PGS's oilseeds.

A Commission spokesman said that the French government did not have the authority to withhold final clearance. The latter stage of the approval process was 'purely administrative', he said. Although EU rules did not specify a deadline, the moratorium clearly violated the spirit of the law, he continued.

Meanwhile, UK government officials are preparing a report for ministers that could lead to a temporary ban on the planting of genetically modified crops. (Extracted from *Chemistry & Industry*, 19 October 1998)

Modified potato withdrawn

Doubts over the effects of genetically modified crops on health and the environment are threatening to undermine attempts by biotech companies to sell them in the European Union.

For the first time, the European Commission's scientific advisers have recommended that a genetically modified plant should be withheld from the market because they cannot guarantee its safety. Britain's environment minister, Michael Meacher, is considering imposing a three-year moratorium on transgenic crops grown for commercial use.

The Dutch company Avebe applied to the Commission for permission to sell a potato that has been modified to produce extra starch. The potato also contains a marker gene which confers resistance to amikacin, an important antibiotic. The Commission's Scientific Committee on Plants, a group of 15 independent scientists, has said the crop should not be licensed for sale in the EU because it was unable to assess the risk of the gene spreading. (Source: *New Scientist*, 17 October 1998)

China to regulate removal of gene resources

The Human Genome Management Office under the Ministry of Science and Technology recently became responsible for regulations restricting the removal of human genetic resources, including human organs, tissues, cells, blood and related materials, from China without the permission of the ministry.

However, the guidelines encourage cooperation with foreign researchers under the principle of shared intellectual property rights.

The office requires a certificate of informed consent from providers of human genetic materials and their families to guarantee the quality of cooperation and protect all participants in genome studies. (Source: *McGraw Hill's Biotechnology Newswatch*, 7 September 1998)

New EC label regulation

A new regulation from the European Council of Industry Ministers governing genetically modified foods in the European Union states that any food product made from genetically modified crops that contain traces of "foreign" DNA or proteins must be labelled accordingly. To avoid labelling, tests must demonstrate the absence of both protein and DNA in the genetically modified foods. The Council proposes to establish a list of products that are exempt from labelling—products including oils and starch hydrolysates that are not considered to contain foreign proteins or DNA. (Source: *Nature Biotechnology*, Vol. 16, July 1998)

Injunction saves Monsanto GM crops from vandalism

In the UK, a renewed High Court injunction has successfully protected Monsanto's genetically engineered crops from a further threat of vandalism.

Served on 18 September 1998, the injunction is one of several blanket attempts to prevent a group called Genetic Snowball damaging trials of genetically modified crops at 60 locations across the UK. The company first took out injunctions against this group in July 1998.

Monsanto moved to reinstate the injunction following threats by the group that it would demonstrate against field trials of genetically engineered herbicide-resistant beet crops near Cambridge. The demonstration, due to take place immediately after the injunction began, failed to occur. An injunction is a court order forbidding individuals or groups doing something which would cause loss or damage to another person or company.

According to Monsanto, the extent of public protest in the UK against field trials of genetically engineered crops is far greater than in any other country. Twenty regulatory bodies around the globe have already approved at least one of the modified crops for commercial growing.

If Monsanto is to develop and market genetically engineered crops in the UK, such as sugar beet and rape seed, it has no option but to conduct trials in the country, said Monsanto. This is because differences in the environmental conditions and the crop varieties grown around the world make local field trials essential to ensure their safety. (Source: *European Chemical News*, 28 September-4 October 1998)

You want to clone? Go ahead

Many laws being introduced to ban human cloning, and some already on the statute books have loopholes that might allow cloners to evade them.

Human cloning by nuclear transfer, the technique used to create Dolly, is not even explicitly prohibited by Britain's 1990 Human Fertilisation and Embryology Act, one of the world's most thorough attempts at regulating human reproduction. "The law bans all kinds of cloning except for the Dolly technique", says Barney Wyld, a spokesman for the Human Fertilisation and Embryology Authority in London. However, he adds that anyone wanting to clone a person would have to apply to the authority for permission—which would be refused.

In the US, legislators have tried to ban cloning at the state and federal levels. California has already passed an anti-cloning law and 21 other states are considering bans. Seven federal bills have been proposed in Congress.

Lori Andrews, an expert in the legal aspects of reproduction at the Chicago-Kent College of Law, says that recent technological advances may make much of that legislation obsolete. For example, at least 11 state bills and California's cloning ban prohibit cloning involving the replacement of a human egg's nucleus with that of another human cell. But researchers in Neal First's laboratory at the University of Wisconsin at Madison have already put the DNA of primates into cows' eggs that have gone on to develop into early

embryos. If the same technique can produce normal human embryos, then these laws could be circumvented.

Other loopholes are created by poor wording. At least eight state bills would prohibit the cloning of a genetically identical person, but Andrews notes that eggs carry mitochondrial DNA in their cytoplasm—so a clone created by nuclear transfer would not have identical DNA.

But even an airtight law could be challenged by Americans who might claim it infringes their constitutional right to reproduce. (Source: *New Scientist*, 9 May 1998)

Ethical issues

EC ethics group established

A new European ethics advisory group with a broadened brief and deeper involvement in the legislative process was established at the end of December 1997 by the European Commission. The European Group on Ethics, Science and Biotechnology, chaired by French lawyer Noelle Lenoir, will help the EC draft new Europe-wide regulations. The body already has to fulfil the roles defined by European legislation, which demands formal ethical assessments in the approval of certain recombinant products, the development of intellectual property legislation, and the instigation of new rules on such controversial subjects as human cloning. The new group shares seven members with the body it replaces, the independent Group of Advisors on the Ethical Implications of Biotechnology. (Source: *Nature Biotechnology*, Vol. 16, February 1998)

AMA decides to wait before passing final judgement on cloning

Delegates at the annual meeting of the American Medical Association voted to delay action on an ethics panel report on human cloning until that report could be coordinated with another committee dealing with scientific affairs.

The report, from the AMA's Council on Ethical and Judicial Affairs (CEJA), had concluded that there was no ethical situation in which cloning a human was acceptable.

At the Chicago meeting, CEJA doctors said the only way cloning technology should be contemplated is in the development of replacement organs or tissues, but that work had to be done without cloning of embryos to produce cells for the replacement tissues.

The committee undertook the report following international controversy surrounding proposed human cloning after British doctors successfully cloned the sheep, Dolly, in early 1997.

The committee reported that "Human cloning as an approach to fertility has ethical hazards in the areas of individual autonomy, privacy and informed consent. It also raises difficult and unresolved legal questions and even more difficult psychosocial problems about family and other social relations. These issues appear unsolvable, and therefore make human cloning an unacceptable treatment for fertility."

In its report, the committee said, "The risk and side effects of cloning have not yet been fully assessed. Absence of knowledge should never be taken in medicine as equivalent to absence of risk."

Cloning for fertility reasons, the committee said, raised numerous risks, including:

- Knowledge of the cloned person's genetic risks;
- The psychological impact of being genetically identical to a parent;
- "New and disturbing motivations" for parenthood.

The CEJA report also renounced cloning as a solution to terminal illness. The report said that even though cloning would produce an identical person genetically, the terminally ill person still dies, while the clone exists separately as do identical twins.

Cloning as an instrument to eliminate genetic defects was also attacked in the report.

It also rejected the notion of cloning foetuses to provide foetal tissues to replace failing organs.

The panel suggested that cloning technology—using somatic cell nuclear transfer to accomplish cell or tissue production—could be used to replace tissues or individual organs without creating human beings.

During testimony in hearings on the report, AMA delegates and other interested parties argued that CEJA and the AMA's Council on Scientific Affairs (CSA) should coordinate efforts to produce reports that were complementary and not contradictory. It was argued that accepting the CEJA report might tie the hands of CSA.

The panels will report their results at the AMA's interim meeting to be held in Hawaii in December 1998. (Source: *McGraw Hill's Biotechnology Newswatch*, 6 July 1998)

HUGO Ethics Committee statement addresses sample collection, sharing

In its February *Statement on DNA Sampling: Control and Access*, the international Human Genome Organisation's Ethics Committee addressed several ethical issues pertinent to sample collection and sharing in genetic research. The committee, which is made up of scientists, ethicists, and lawyers from ten countries, also confirmed its commitment to the principles of its March 1996 *Statement on the Principled Conduct of Genetic Research* (hugo.gdb.org/conduct.htm).

The ethics group made the following recommendations regarding DNA sampling:

- Choices offered in the consent process should reflect the potential uses of the DNA sample and its information.
- Routine samples obtained during medical care may be used for research if there is general notification of such a policy, the patient has not objected, and the sample has been coded or anonymized.
- Research samples obtained with consent may be used for other research if the conditions in the statement above are met.
- Security mechanisms must be initiated to ensure respect for the choices made and the desired level of confidentiality.
- Special considerations should be made for immediate relatives, who should have access if there is a high risk of having or transmitting a serious disorder and if prevention or treatment is available.
- Stored samples may be destroyed at the request of the person if immediate relatives do not need access.
- Except as authorized by law, no disclosure of research participation or results should be made to institutional third parties without appropriate consent.
- International standardization of ethical requirements for control and access of DNA samples and information is essential.

(Source, *Human Genome News* 9 (3), July 1998)

Ethical measures to boost biotech

The German cabinet has adopted a range of measures aimed at improving the competitiveness of the country's biotechnology industry while setting strict ethical limits.

The measures, recommended to the cabinet by the government's technology council, would make it easier for German scientists to perform genetic experiments. Researchers would no longer have to seek permission to perform experiments that are considered to involve no risk. The government would also work with the food industry to create clear laws governing the export of genetically modified food, a move designed to improve public confidence.

The proposals would impose a strict ban on cloning humans. The alteration of genes in human egg cells and the creation of human embryos for research purposes would also be forbidden. In addition, the government would set up a DM1M/a bioethics centre to be run in conjunction with the German Research Association.

Biotechnology is one of the new industries that the German government is promoting as a solution to the country's growing unemployment problem, but the public remains largely opposed to biotechnology and Germany has filed fewer biotech patents than many of its European competitors. (Source: *Chemistry & Industry*, 2 February 1998)

Biosafety

Friend turns to foe

Bacteria sprayed onto crops to kill pests can also harm people under certain circumstances. Microbiologists in France have treated a soldier who developed a serious wound infection caused by *Bacillus thuringiensis*.

B. thuringiensis produces an insecticidal toxin, and so has become popular as a "natural" pesticide. Its role as a human pathogen emerged when a team at the military hospital in Saint-Mandé, near Paris, examined the wounds of a soldier injured in 1995 by a mine at Sarajevo airport in Bosnia.

Initially the bacterium infecting the wounds was identified as *Bacillus cereus*, but unusual crystals in the cells prompted the team to have the strain rechecked. Laboratories at the WHO and at the Pasteur Institute in Paris independently identified the sample as *B. thuringiensis* serotype H34-*konkukian*.

The researchers put the same strain into wounds in mice with weakened immune systems. The bacteria caused a nasty infection—but only if they had first been grown in a medium containing blood. Exposure to blood seems to switch the bacteria into a pathogenic form.

Farmers spray crops with the strains H1, H2 and H3, rather than the H34 serotype. But more recent work by the French team has shown that one of the commercial strains can also infect wounds in mice. (Source: *New Scientist*, 30 May 1998)

E. coli declared hazard group 3 in UK

The food poisoning bacterium *Escherichia coli* O157 is now officially as dangerous as the agents that cause anthrax and rabies.

Alarmed by the plight of six microbiologists who became seriously ill in 1996 after working with *E. coli* O157, Britain's Health and Safety Executive (HSE) has pushed the European Union to introduce stricter safety rules for anyone working with the bacterium in the lab. They must come into effect by 30 June 1998.

E. coli O157 has been raised from a hazard group 2 pathogen to hazard group 3. This means that the bacterium now commands the same degree of caution as the pathogens that cause anthrax, rabies, AIDS, tuberculosis, typhoid fever and BSE. This is one level below the most dangerous classification—hazard group 4—which includes the viruses that cause Ebola, Lassa fever and smallpox.

Other strains of *E. coli* remain in hazard group 2, which also includes most strains of *Salmonella*, viruses such as *Herpes simplex* and the adenoviruses and rhinoviruses that cause common colds.

The new rules will affect laboratory work on *E. coli* O157 in two main ways. Workers at intermediate risk are clinical microbiologists in hospitals and abattoirs who regularly analyse faecal samples to test for the bacterium. From now on, they will be required to work in a separate room from their colleagues.

The researchers at highest risk are those who routinely culture the bacteria and work with it. They must now operate in separate purpose-built laboratories. The lab must have an inward air flow to prevent the escape of any airborne *E. coli* O157. Researchers must use sealed glove boxes whenever they work with aerosols containing the bacteria. (Source: *New Scientist*, 23 May 1998)

Biosafety assessment of the GUS reporter gene

The B-glucuronidase (GUS) gene is to date the most frequently used reporter gene in genetically engineered plants. As such, it is likely to be present in some of the transgenic food, feed and ornamental crops being developed for commercial use. To aid the biosafety evaluation of GUS-containing transgenic crops, the ecological and toxicological aspects of the gene and gene product have been examined. The assessment concluded that GUS in genetically modified plants and plant products can be regarded as safe for the environment and consumers.

Biosafety assessments generally consider whether a transgenic organism presents an ecological and toxicological concern. These concerns include whether the modified crop may have a selective advantage or may become a weed; whether the transgenic character may spread from the crop to wild relatives or other organisms that as a result become problematic or may somehow disturb ecological relationships; and whether the introduced gene products are toxic or harmful to humans or other organisms. The assessment of the GUS reporter gene dealt with the origin, biological function and substrate specificity of the GUS enzyme. The work, conducted by a team of researchers from the Department of Molecular Biology, University of Wageningen, the Netherlands and supported by three Ministries within the Dutch government, was chaired by Prof. Dr. P. G. de Haan.

GUS activity is found in many bacterial species. It is also common in all tissues of vertebrates, and is present in organisms of various invertebrate taxa. Much debate has occurred with respect to the presence of GUS or GUS-like activity in plants. If any, such activity is low and its putative functions are unknown. Thus the gene is particularly useful as a reporter in transgenic plants because its activity can be detected in the absence of a background level of endogenous GUS expression. The gene used in transgenic plants originates from the enterobacterial sp. *E. coli* that is widespread in the vertebrate intestine and in soil and water ecosystems.

In the field, the question becomes whether a GUS-expressing plant or its progeny has a selective advantage compared to the untransformed parent plant due to expression of the reporter gene. Substrates for GUS activity could be present in the soil as a result of manuring, and could be taken up by plants. It is conceivable that in contrast to unmodified plants, the GUS-containing plants would be able to metabolize these compounds. The metabolites conceivably

could be able to influence plant performance in a different manner than the intact substrate would influence the performance of non-transgenic plants.

This scenario is not likely for several reasons. First, any substrate in the field would be rapidly (within hours) hydrolysed by GUS activity from bacteria present in the manure. This would result in a low concentration of substrate in the soil. Another possibility is that the transgenic GUS enzyme is released from genetically modified plants. The leaked enzyme must then compete for substrates with the analogous enzyme naturally present in soil bacteria. This situation will not change the environment around such plants significantly. Therefore plants expressing GUS transgenes will not have any selective advantage in the field, and any GUS activity added to the ecosystem through genetically modified plants would be negligible.

A second issue related to genetically modified plants is the possibility of making the plant more weed-like. The biosafety evaluation concluded that neither GUS-expressing crop plants nor wild relatives that might acquire the transgene through outcrossing would be expected to exhibit any increase in weediness. Weediness is the result of many different independent characteristics. Most crop plants lack several or most weedy traits. Expression of GUS in plants is not likely to alter or add any weedy characteristics to any crop plant. A GUS-containing plant does not exhibit ecological characteristics that distinguish it from the untransformed plant, apart from having GUS activity. Data from numerous field tests support this view.

Toxicity of GUS in food from genetically modified plants is not a concern. The enzyme in transgenic foods is identical to the GUS from *E. coli* in the digestive tract, and homologous to the endogenous GUS from epithelial cells of the intestine. Both types of enzyme are ubiquitously present in the gut, therefore consumers are continuously exposed to GUS. In many fresh foods such as raw beef and oysters, GUS is abundantly present and enters the digestive tract of consumers without harmful effects. The widespread occurrence of GUS and the constant exposure to the protein also preclude all concerns about any putative allergenicity.

This biosafety assessment is expected to contribute to informed decisions about the release of GUS-containing crops. L. J. Gilissen, L. J. W., P.L.J. Metz, W. J. Stiekema and J. P. Nap. 1998. Biosafety of *E. coli* B-glucuronidase (GUS) in plants. Transgenic Research Vol. 7, No. 3, 157-163. P. Janaki Krishna, Biotechnology Unit, Institute of Public Enterprise, Hyderabad, India ipe@hyd.ap.nic.in (Reproduced from ISB News Report, July 1998) (Source: *Australasian News*, Vol. 8, No. 4, August 1998)

New global studies reveal unabated loss of biological diversity

The 1997 IUCN Red List of Threatened Plants, a landmark international survey conducted as a 20-year joint effort by 16 research organizations, finds that at least one of every eight known plant species on Earth is threatened with extinction. Approximately 34,000 species could disappear, and some entire plant families are endangered, according to the report, the first-ever comprehensive, global list of threatened plants, edited by Kerry S. Walter and Harriet J. Gillett. As more than one half of all prescription drugs are modelled on natural compounds and one fourth are made directly from plants or chemically modified plant substances, widespread extinction of plant species would drastically affect medical science, according to the experts involved in the project. With about 50,000 plant species yielding about 50 drugs, the anticipated loss of 34,000 species could mean the loss of 34 new pharmaceuticals. The World Conservation Union (IUCN), in cooperation with the Smithsonian Institution, the World Wildlife Fund (WWF), the Nature Conservancy, the Royal Botanic Gardens at Kew and Edinburgh, and ten other research groups published the massive 862-page report. Since information from Asia, Africa, and South America is often incomplete, the real situation could be even more serious than the report indicates, reporters were told at a Smithsonian press briefing. For information, contact: IUCN Publications Services Unit, 219c Huntingdon Rd., Cambridge CB30DL, UK. Tel.: +44-1223-277894; Fax: +44-1223-277175; e-mail: IUCN-psu@wcmc.org.uk or: Ricardo Bayon, IUCN-UD. Tel.: +1-202-797-5454; Fax: +1-202-797-5461; e-mail: bayon@iucnus.org

Another recent survey conducted by the Center for Plant Conservation (CPC), *Usefulness and Economic Potential of the Rare Plants of the United States: a Statistical Survey* by Oliver L. Phillips and Brien Meilleur (published in Economic Botany, 52 (1), January-March 1998, pp. 57-67) indicates that losses of rare wild plants in the US represent a substantial economic threat. The survey shows that endangered food crop relatives alone have an estimated worth of about $10 billion annually in wholesale farm values. Many crops that are important globally require periodic genetic infusions from wild relatives to combat climatic change or evolving disease, according to the report, and plants were essential in the development of seven of the 20 best-selling pharmaceuticals in the US. The study was supported by the Surdna Foundation and Phillip Morris Inc./Kraft Foods, with assistance from the Missouri Botanical Garden. The CPC, headquartered at the Missouri Botanical Garden in St. Louis, Missouri, states that it is the only national organization with the sole aim of preventing the extinction of native plants of the U.S. It coordinates 28 botanical gardens and arboreta that maintain rare native plants for research purposes and to prevent their extinction. For further information, contact: Brien Meilleur, Center for Plant Conservation, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166-0299. Tel.: +1-314-577-9451; Fax: +1-314-577-9465; Internet: http://www.mobot.org/CPC (Source: *Diversity*, Vol. 14, Nos. 1 and 2, 1998)

General

First commercial European maize plantings

For the first time in Europe, the commercial planting of genetically engineered maize varieties began in April in Germany. Novartis Seeds' (Basel, Switzerland) BT176 variety contains a triplet of genes encoding *Bacillus thuringiensis* (*Bt*) toxin, resistance to the Basta herbicide, and, controversially, resistance to the antibiotic, kanamycin. However, only the *Bt* gene is expressed. The product was approved for import into the European Union in December 1996. Although registration for commercial planting in France and Spain was granted early this year, Novartis has not applied for registration in Germany. However, the Federal Variety Office (*Bundessortenamt*; Hannover) allows "premarketing" of 10 tonnes of the recombinant crop to a limited number of wholesalers. According to a spokesperson for Novartis Seeds (Bad Salzuflen, Germany), one tonne of transgenic Bt-maize seeds has already been delivered to farmers in Germany and has probably been sown. This quantity would seed just a fraction (350 hectares) of the 1.6 million hectares of maize grown in Germany. Farmers are not obliged to mark fields of

BT176 nor to disclose sites in publicly available documents. (Source: *Nature Biotechnology*, Vol. 16, June 1998)

Growing problem in seed banks

Many of the world's ancient crop seeds are being contaminated or lost by the seed banks that are charged with preserving them, research by German scientists suggests.

Adolf Steiner and colleagues at the University of Hohenheim examined samples of oats dating from 1831. The oats had been recovered in 1956 from a demolished theatre in Nuremberg and stored at seed banks in Germany and Austria. Since then they have been grown, harvested and returned to storage several times, as seeds must be to maintain their viability.

The scientists analysed the proteins in samples of the oats from each bank. They found that most of the 28 samples had one or two patterns of proteins, showing that the original oats belonged to two strains. But 12 of them had unusual protein patterns, implying they had been contaminated either by oats of the other Nuremberg strain, or by foreign oats.

The losses, says Steiner, were "simply due to carelessness during harvesting or handling". The team is finding similar contamination in samples of spelt wheat, once the most common grain in Europe which almost disappeared this century. Such old varieties, says Steiner, "are vital for breeding new crops".

He fears that the main reason for the disruption is a lack of funding, and that seed banks worldwide could be suffering the same problems. Reharvesting seeds is the most expensive work a seed bank does. (Source: *New Scientist*, 14 February 1998)

Bioscience business blasts off

Europe's biotechnology sector is booming, according to the latest industry survey.* The sector's growth rate more than doubled in 1997 with the number of companies rising 45 per cent, employment increasing 42 per cent and research and development spending going up 27 per cent. This year is likely to be another year of robust growth.

The survey, which is the sixth in an annual series published by consultants Ernst & Young, shows that the UK is still Europe's leading biotech nation with around 250 firms. Germany has the continent's fastest-growing sector, almost doubling its number of firms over the year to 175.

Europe now has a total of 1,036 biotech companies employing over 39,000 people and spending Ecu 1.9 billion per annum on research and development, the report concludes.

But the report highlights a few problems. Public fund raising fell 68 per cent to Ecu 450 million as setbacks in the UK sector forced companies to postpone flotations. Only 61 of Europe's companies have a stock exchange listing. The survey also points out that the industry has yet to launch a blockbuster product. All 11 "pure" biotech drugs approved in 1997 by the European Medicines Evaluation Agency were made by US companies or large multinationals.

Nevertheless the availability of private finance rose 50 per cent to Ecu 385 million and European companies are expected to launch major products 'in the next year or two'. (Extracted from *Chemistry & Industry*, 4 May 1998)

*"European Life Sciences 98: Continental Shift", Ernst & Young, Becket House, 1 Lambeth Palace Road, London SE1 7EU, UK.

Farmers may soon be entirely reliant on seed companies

Terminator technology: that is what farmers are calling a breakthrough in genetic engineering designed to prevent the seeds of agricultural crops from germinating. They fear it could spell the end of the tradition in poorer countries of saving seed from one season's crop to replant the next.

The US Department of Agriculture (USDA) and a Mississippi seed firm, the Delta and Pine Land Company, were granted a patent for a technique that can sterilize the seeds produced by most agricultural crops. They expect the technique to be adopted within the next five years by all the major seed companies, which have been looking for ways to prevent farmers from recycling seeds from their crops for many years.

"It's terribly dangerous", says Hope Shand of the Rural Advancement Foundation International, a pressure group based in Canada. "Half the world's farmers are poor and can't afford to buy seed every growing season. Yet they grow 15 to 20 per cent of the world's food."

The technology depends on a promoter sequence from a gene called *Late Embryo-genesis Abundant (LEA)* that activates the gene to which it is attached only when the plant's seeds are maturing. The researchers attached the *LEA* promoter to a gene that produces a protein which prevents germination. They inserted this into seeds. At the end of the growing season, the promoter switches on this gene.

Melvin Oliver of the USDA's labs in Lubbock, Texas, who invented the technique, claims that seeds manipulated in this way will grow into healthy plants that produce sterile seeds. He anticipates that it will be welcomed by seed companies, who regard the replanting of seeds as theft of their intellectual property. (Source: *New Scientist*, 28 March 1998)

Transferring EU-funded biotechnology research to European bioindustry

The European Union (EU) is currently deliberating the content and budget for its next scientific research and development programme, Framework 5. This will begin in 1999 and, under current proposals, life sciences research will have a budget of over Ecu 2.5 billion ($2.8 billion). However, the results of a recent survey of both research groups and companies suggest that a good deal of EU-funded biotechnology may be falling down gaps in some of the most basic aspects of the technology transfer process.

The uptake of EU-funded biotechnology research by European bioindustry is a stated aim of the European Commission's 4th Framework Programme (1994-1998). A key mechanism through which the EU tries to achieve this aim is the Innovation Programme, a scheme established in 1995 and controlled by the Directorate for Telecommunications, Information Market and Exploitation of Research of the European Commission (DG XIII). It aims to be an interface between the market and the various EU research programmes and has an overall budget of over Ecu 250 million for the period 1995-1998 (inclusive). Perhaps the most startling finding of the recent survey is that most companies—from large pharmaceutical companies to small or medium-sized enterprises—know nothing about the Innovation Programme or value it little. Of 95 European companies surveyed, 66 per cent had not heard of the Programme. Of those that had, 65 per cent did not think it effective.

It was not as if the companies were uninterested in Europe or in biotechnology: They were all members of the European Association of BioIndustries (Brussels).

Furthermore, the respondents were keen on technology transfer. Two thirds of the sample (65 per cent) supported European collaborations in technology transfer, and 60 per cent supported European collaboration in research and development. There was a unanimous desire, too, among the companies to be kept informed of technical opportunities arising from EU-funded biotechnology research.

The survey also investigated attitudes regarding technology transfer among 135 researchers (a 70 per cent response) from 14 countries who were funded under the 4th Framework Biotechnology Programme. They clearly recognized the central role of obtaining intellectual property rights (IPR) in the results of their work: 86 per cent considered that protecting their results was important. However, a majority of all researchers (56 per cent) stated that they needed technical assistance in IPR, suggesting that many European universities and research organizations are falling short of researcher expectations and needs. One way to deal with this would be to establish a concerted European action/network to offer institutions—especially those without licensing departments—assistance on various aspects on IPR and technology transfer such as technology evaluation to company negotiations. One obvious model for such an umbrella association is the Association of University Technology Managers, which assists universities in transferring their technologies to the market place.

The survey has highlighted that IPR assistance to European biotechnology researchers and an efficient communication interface to bring all EU-funded biotechnology research to the attention of European bioindustry are still needed.

A sound governmental support framework on a European basis is necessary to reinforce the technology transfer process between EU-funded biotechnology researchers and bioindustry, according to the survey. Adding value to basic research by converting discoveries into inventions and ultimately products is a process that evolves in a more targeted manner. By facilitating the access to European bioindustry of new scientific technologies arising from EU-funded biotechnology research, the competitiveness of Europe in sectors such as human health, agriculture, and environmental protection will ultimately be increased.

Colm Lawler is project manager of BioResearch Ireland (lawlerc@biores-irl.ie); Robert van der Meer is director of the Netherlands Industrial Agricultural Biotechnology Association (niaba@xs4all.nl); and Jacques Viseur is director of Eurotop Co-operation Partners Brussels (esnba@interpac.be).

C. Lawler, R. van der Meer, and J. Viseur, May 15, 1998. The survey was 50 per cent financed by the European Commission under the 4th Framework Biotechnology Program, 2nd Call. Contract No. ERBIO4 CT 960387. The authors acknowledge the support of the European Association of BioIndustries (EuropaBio) in conducting this survey. (Source: *Nature Biotechnology*, Vol. 16, June 1998)

HIV devastates a continent

Just five years ago, babies born in Zimbabwe had a life expectancy of 61 years. Many could expect to live to see their great grandchildren born. Today, thanks to AIDS, their life expectancy is 39 years.

These devastating figures have emerged from a new study by the US Census Bureau, which has for the first time calculated the impact of the disease on life expectancy across Africa. The study reveals a demographic holocaust in which population growth in most countries is being slashed. It marks a return to the conditions of the last century, when high birth rates were neutralized by equally high death rates.

Zimbabwe is the worst case, with a quarter of adults infected with HIV. Death rates are "three times higher than they would have been without AIDS", says the report. Most of the dead are young adults and infants infected by their mothers. The country's annual population growth rate has fallen from around 3 per cent in 1992 to 1.1 per cent today. By 2010, newborn Zimbabweans will have a life expectancy of just 31 years.

Other countries are not far behind. The report, *Focus on HIV/AIDS in the Developing World* by Peter Way and his colleagues at the Census Bureau, reveals that in Kenya, life expectancy has been slashed by 18 years to 48 years, and in Botswana by 22 years to 40. One of the few rays of hope is Uganda, where prevention programmes are starting to reduce infection rates.

In September, the United Nations Development Programme warned that anybody who thinks this brake on population growth might promote African economic development is seriously mistaken. "HIV/AIDS is having a significant impact on economies, creating shortages of skilled labour", the agency noted. (Source: *New Scientist*, 17 October 1998)

New AIDS threat

A new strain of HIV has been discovered in Cameroon. In the September issue of *Nature Medicine* (Vol. 4, p. 1032), François Simon of the Bichat Hospital in Paris and his colleagues describe the virus as a genetic intermediate between the common group M strains and the rarer group O.

The new virus, designated group N, might not be picked up by existing antibody tests for HIV. However, Simon Wain Hobson of the Pasteur Institute in Paris predicts that group N infections will remain rare. (Source: *New Scientist*, 5 September 1998)

New "Terminator" technology threatens farmers' rights

Zeneca, the life industry spin-off of the old ICI (Imperial Chemical Industries), has stated that it will apply for patents in 58 countries for its invention that renders it impossible for farmers to save "protected" seed from growing season to growing season.* This new technology could produce, for example, genetically engineered seed that would not germinate unless exposed to Zeneca's private chemical trigger, or plants genetically programmed to become stunted, not properly reproduce or not resist disease(s) unless sprayed with Zeneca's chemical formula.

The move by the British firm is hard on the heels of the US patent granted in March to the US Department of Agriculture (USDA) and Delta and Pine Land Company for what the Rural Advancement Foundation International dubbed "Terminator Technology". Within weeks of that patent

*This new technology has been nicknamed the "Verminator" technology by RAFI. In the patent description, Zeneca described the source of one such "killer" gene as coming from "mammalian uncoupling protein isolated from the brown adipose tissue of Ratus ratus".

For background on these trends and on the activities of the global seed trade please visit RAFI's homepage at www.rafi.ca

announcement, the US agrochemical corporation Monsanto bought Delta and Pine for US$ 1.76 billion. Zeneca is currently the world's fifth largest seed company with annual sales of US$ 437 million in 1997. It is also an important crop chemical and drug company.

The Food and Agriculture Organization of the United Nations estimate that 1.4 billion poor people depend on farm-saved seed for their food security. The farmers involved often grow their food under unfavourable conditions of little commercial interest to global seed companies. Thus, farmers adapt or breed their own varieties that meet their own conditions and needs. These new technologies can make it impossible for farmers not only to save seed but to create the varieties they need.

RAFI's research director, Hope Shand has been tracking the Terminator Trend for some time. "It's not just these two technologies", Shand asserts, "Monsanto and Pioneer are developing new wheat hybrids they believe can take over the market". Second-generation seed will either not breed true or it will be sterile. Until recently small grain cereals such as wheat and rice were difficult to commercially hybridize. "Now, that seems to be changing", says Shand, "The opportunity to force farmers back to buy seed every season has led multinationals to focus on hybrid terminators too". (Source: *Global Pesticide Campaigner*, Vol. 8, No. 3, September 1998)

Monsanto buys seed business

US life sciences giant Monsanto is expanding its genetically modified crop operations across four continents with the $1.4 billion acquisition of Cargill's international seed business.

The deal includes seed research, production and testing facilities in 24 countries, and sales and distribution outlets in 51 countries across Latin America, Europe, Asia and Africa. The deal does not include Cargill's operations in the US and Canada, nor Cargill Agricultural Merchants in the UK.

This deal follows an earlier agreement between Monsanto and Cargill to form a global joint venture to create and market products for the grain processing and animal feed markets. The two companies are also exploring other ventures in food and agriculture.

The deal strengthens Monsanto's position against rival biotech firm Pioneer Hi-Bred International in countries such as Argentina and Brazil. Pioneer, which is closely allied with DuPont, also has a strong presence in Europe.

Minneapolis-based Cargill specializes in the development and marketing of corn, sunflower and rape seeds. It also markets soya bean, alfalfa, sorghum, wheat and hybrid rice seed. (Source: *Chemistry & Industry*, 6 July 1998)

Genomics at heart of revolution in corporations, global economy

Genomics, the study of genetic sequences, is sparking a major restructuring of some of the world's largest companies and a revolution of the global economy, a Harvard University economist said.

Genomics is leading to novel molecules that are biologically important and that blur the traditional boundaries between pharmaceuticals, biotechnology, agriculture, chemicals, environment, computer and other industries. That in turn is causing the world's largest companies to restructure in a way that will change the world's economy and create a new life science industry.

Juan Enriquez, a Harvard University economist, said genomics already is driving megamergers among companies that want to lock in patents and licensing agreements.

The largest pharmaceutical company merger so far, that of Swiss drug makers Ciba-Geigy AG and Sandoz AG, created a $100 billion conglomerate called Novartis that has enough money and R&D capability to compete in the health care, nutrition and agriculture businesses.

Pharmaceutical companies also increased alliances with other companies sixfold between 1993 and 1996. Enriquez said genomics differs from the biotechnology industry of the 1980s, which gained interest from Wall Street traders and venture capitalists by promising new treatments, but delivering fewer than anticipated.

He said biotechnology companies tended to act alone from development of a molecule through its commercialization. This kept them relatively focused on certain drug areas, and thus also kept the companies small.

Technology also is playing a major role in the breadth of complementary technologies in the genomics business. Now researchers can analyse hundreds of thousands of compounds at the same time rather than being limited to looking at just a few genes at a time. Enriquez predicted that future mergers increasingly will take place outside of a company's traditional industry.

Current stock market valuations of life science companies are very high. To meet the short-term earnings expectations of investors, some conglomerates will have to create a series of blockbuster products quickly, and that could result in the same roller-coaster financial cycle of 1980s biotechnology companies. But because of their size, conglomerates with earnings gyrations could have a more substantial impact on international stock markets than did small biotechnology concerns.

In addition, the pressure to introduce genetically engineered products may cause companies to push out products before they are ready. And discoveries and company restructurings are moving much faster than public comprehension of the industry.

Enriquez said life science companies will have to cooperate more with foreign counterparts and adhere to rigorous product standards. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 17 August 1998)

Mycogen/R-PR in jv

Mycogen and Rhône-Poulenc Agro, the crop protection subsidiary of Rhône-Poulenc, are forming a worldwide plant biotechnology alliance.

The firms will pool plant biotechnology assets to develop and market genetically modified plants and seed products containing multiple traits. The collaboration would initially focus on modifying cotton and sugarcane with Mycogen's insect resistance gene derived from the natural insecticide *Bacillus thuringiensis* (Bt) and Rhône-Poulenc Agro's gene sequences which provide tolerance to herbicides, including glyphosate, bromoxynil and isoxazoles.

The proposed agreement also provides for future expansion of the alliance to develop insect-resistance and herbicide-tolerance traits for other crops, including corn, canola, soybean and sunflower, and to incorporate additional agronomic and quality traits such as oil and protein output enhancement.

Mycogen and Rhône-Poulenc Agro plan to market the jointly developed plants with multiple traits through licenses to seed companies worldwide. In North America, South

America and Europe, Mycogen would also market resulting seed products directly through its seed companies and affiliates. The US cotton and Latin America sugarcane markets will be the initial focus of this new alliance. (Source: *European Chemical News*, 10-16 August 1998)

Malaria immunity raised by vitamin A and zinc

The malarial parasite, *Plasmodium*, is one of the deadliest and most devious on earth. As it wends its way from mosquito to man, it causes fever and damage to the spleen in perhaps 500 million people a year. Of these, it kills an estimated 2.7 million—most of them children under five.

Unlike the disease itself, remedies for malaria are decidedly scarce. There is no foolproof vaccine, and the microbes, as well as the mosquitos that carry them, have evolved resistance to once-powerful anti-malarial compounds. Yet researchers at Johns Hopkins University in Baltimore, Maryland seem now to have discovered a cheap and easy fix. Anaruj Shankar and his colleagues have found that feeding zinc or vitamin A to children seems to raise their immunity to malaria.

In a 13-month trial that he conducted in New Guinea, which involved feeding almost 500 children vitamin A every three months, Dr. Shankar found that he could reduce the incidence of the disease by 30 per cent and in a ten-month study of over 270 children, he found that those receiving ten milligrams (mg) of zinc a day had 40 per cent fewer attacks of severe malaria than those on normal, low-zinc diets. This was probably because the children taking extra zinc produced more biochemicals that affect the immune system, such as interferon-gamma.

Sadly, neither zinc nor vitamin A is as good at preventing disease as are anti-malarial drugs such as mefloquine. On the other hand, they are not so expensive. A year's worth of each of the two supplements costs roughly a dollar per child. A year's supply of mefloquine costs 50-100 times that. And it may, in any case, be possible to get results that are closer to those of drugs by combining the two food supplements.

To find out whether the effects of zinc and vitamin A are, indeed, complementary, Dr. Shankar is planning to start a further trial in Ghana. And to see if food supplements can boost the effects of drugs, an international study (organized by USAID and Harvard University) is due to start looking at the combined action of zinc and anti-malarial drugs in 1,200 children in Ghana, Uganda, Tanzania, Ecuador and Zambia in October. (Extracted from *The Economist*, 1 August 1998)

Explosive growth is projected for DNA chips

As life sciences become increasingly competitive and lucrative, the requirement for technical capabilities in the field of genomics, the study of gene structure and function, becomes paramount for companies that need more efficient and accurate methods for drug discovery and development. Part of the need for genomics capabilities involves the rapidly emerging field of bioinformatics, which involves obtaining, storing and analysing information derived from studying biological systems. Crucial to bioinformatic analysis are computer algorithms that can compare newly isolated genes with databases containing genetic sequences of known function.

Methods of experimentation used to obtain information for bioinformatic analysis include electrophoresis, chromatography and a relatively new area, biochips. Biochips, which include DNA chips, protein chips and lab chips, are miniaturized devices that can make biological experimentation more efficient. They contain either immobilized DNA strands (DNA chip), immobilized protein strands (protein chip) or interconnected channels with fluid propulsion and control systems etched into glass, silicon, quartz, or plastic (lab chip) that respectively permit gene, protein and expression system analysis on a single chip.

With the cost of new drug development estimated between $300 and $500 million, there is strong demand for techniques, such as biochips that can be used to accelerate drug discovery and development. The biochip market is expected to balloon from a 1997 level of $12 million to $632 million in 2005, according to Foster City, California-based Front Line Strategic Management Consulting, which recently completed a study on the biochip market.

DNA chips (also referred to as gene chips or DNA microarrays) now dominate the biochip market, accounting for 94 per cent of the expected $42 million worth of biochips sold for 1998, according to Front Line. The market is expected to achieve a compound annual growth rate of 77 per cent between 1998 and 2001, with the primary applications for drug discovery, drug target identification, pharmacogenomics and agricultural biotechnology.

Although now occupying relatively small positions in the biochip market, protein and lab chips are two emerging market segments. As protein chips become more established, their market share is expected to increase from a current level of 6 to 10 per cent in 2005, according to Front Line.

Protein chips, which consist of immobilized protein strands, provide information that is used in pathway/methanism research, determining drug toxicology and efficacy screening. Proteonomic analysis is a method that can be used to interpret data derived from experiments using protein chips by using computers in a way similar to bioinformatics, but involving amino acid sequences as opposed to nucleotide sequences.

Lab chips, which can provide a miniature surface for processing a large number of biochemical experiments, are expected to hit the market in 2000. Front line projects that the lab chip market will grow rapidly and capture a 10 per cent share of the biochip market by 2005, due to heavy demand to fully integrate, automate and miniaturize research laboratories. Lab chips will also fill the need to prepare samples for DNA and protein chips and for faster and more efficient combinatorial chemistry. Direct applications will include diagnostics and forensics.

The full potential of biochips, whether DNA, protein or lab chips, is realized in the broader context of bioinformatics, where software applications and related hardware systems are used to manage and organize the data generated from biochips. (Extracted from *Chemical Market Reporter*, 9 November 1998)

Growing market for botanicals

A growing international market for herbal ingredients for pharmaceuticals, dietary supplements and cosmetics is broadening the sourcing of plants around the globe. At the same time, greater volumes of known plants are being harvested in more countries, and this is accelerating the search for new compounds. This is placing increased demand on botanical suppliers and firms involved in natural products, which must navigate sourcing issues with local governments as well as increase internal cultivation of plant sources.

One example of a company confronting these issues is Shaman Pharmaceuticals, a South San Francisco-based firm

specializing in natural product drug development. "We're in 30 countries now", says Stephan King, executive vice president of ethnobotany and conservation at Shaman Pharmaceuticals. Shaman has publicly raised and invested over $100 million during the past decade, working with local healers or shamans and governments for drug discovery and development. A promising candidate is Shaman's Provir, a drug based on latex from the croton tree, which is found throughout South America. The drug is in phase III clinical trials for the treatment of diarrhoea associated with AIDS.

Before Shaman focused on its efforts to bring Provir to market, the company put in years of groundwork in many of the botanicals sourcing hot-spots around the world.

INBio, the National Biodiversity Institute, is a Costa Rica-based agency that is setting a global trend among government entities through the collection and cataloguing of plants and other natural species in the forests of Costa Rica. But the work of contacting a local shaman, working with local indigenous tribes, and establishing a legal structure with the approval of the sovereign government can be arduous even in such a progressive country.

On the other hand, countries with great potential as sources of botanicals, such as Brazil, have yet to finalize their legislation regulating botanicals prospecting, so companies like Shaman have steered clear of the country after several unsuccessful attempts to set up sourcing arrangements.

A major question in agreements for sourcing botanicals is how much royalty is paid to the local and government partners, and when. Shaman is dividing a percentage of profits among the thirty countries they work in, giving half [of the country total] to the government agency with responsibility for the conservation of biodiversity, and half to the cultural groups involved—which may be one or four different groups.

One issue that is not readily disclosed is the exact value of these donations. "The percentage that we donate is proprietary until we've done it; revealing it [too early] weakens negotiations", Mr. King says.

Trusts are one measure of financial commitment, however. "We recently set up a $40,000 Nigerian trust fund, which became a case study. Trust funds are the way of the future, but they must be transparent, with visible boards representing the shamans, healers and the government", he says. "In the past, local groups have not been included [in other companies' agreements]. But we are interdependent on the healers and shamans", he adds.

Multi-level tribe-plus-government arrangements are necessary in Latin America and Africa, where the use of medicinal plants in society is less organized than in Asian countries.

In China, the use of botanicals in medicinals is so advanced that herb shops routinely offer customers sophisticated mixtures of botanicals to treat an illness. The chemical action of the individual plants within these mixtures is often much milder than that of a single-plant-supplement used in Western cultures, he notes.

Some companies such as Pharmagenesis, a Palo Alto, Californian-based firm specializing in medicinal plant chemistry, see China as the key to building a global business in botanicals. Pharmagenesis focuses on immunology and hematopoiesis—the process in which bone marrow converts stem cells into platelets and white blood cells—and expects to have a drug on the market in China within a year.

Other companies are focusing on China and the rest of Asia as a source for botanicals rather than as an end market.

The success of pharmaceuticals, such as Taxol, and of dietary supplements, such as St. John's wort, has led major pharmaceutical and vitamin companies to step up their consumption of botanicals markedly. (Extracted from *Chemical Market Reporter*, (13 July 1998)

C. COUNTRY NEWS

Australia

Micro-plants yield pharmaceutical new wave

Compounds with anti-cancer properties and potential for use in new generation antibiotics and nutritional supplements have been found in tiny marine plants around Australia's coastline.

Microalgae—single-cell marine plants at the base of the ocean food chain—produce a range of biochemicals with exciting potential, say researchers. Australian microalgae are genetically and biochemically different from microalgae found in oceans elsewhere, says Dr. Susan Blackburn of CSIRO's Collection for Living Microalgae.

Dr. Blackburn said the two year project is part of a national collaborative super-project called the Bioactive Molecule's Initiative involving six CSIRO Divisions, encompassing the strengths of CSIRO biotechnology with a major focus being marine biotechnology. The recent discovery of these biologically-active compounds in certain Australian microalgae highlights the potential for a new biotechnology dimension for Australia's oceans, she said.

The CSIRO collection for Living Microalgae holds over 700 species of microalgae in the collection, which is the largest of its type in Australia and one of the largest in the world. Dr. Blackburn estimates there could be thousands of different microalgae in Australia's ocean territories which, at 16 million square kilometres, cover twice the size of the nation's landmass. Microalgae from the collection is presently used throughout Australia in the formulation of crucial live feeds for young aquaculture species such as oysters, prawns and abalone.

The human health benefits of microalgae are a relatively new development, with only about three microalgae species being cultured for nutriceutical production internationally, supplying large markets in Asia and America. Australian manufacturers are also supplying the nutriceutical market with betacarotene and other compounds derived from microalgae.

More information from: Dr. Susan Blackburn, Tel.:03 6332 5307 or Katherine Johnson, Tel.: 03 6232 5113. (Source: *Australasian Biotechnology*, Vol. 8, No. 4, August 1998)

DNA testing joins fight against illegal trafficking of wildlife

Technology is set to become the central tool in the fight against the illegal trade of Australian wildlife. A new DNA typing laboratory and databank is being set up at Queensland University of Technology's School of Life Sciences in Brisbane. Over the next 18 months, under the direction of the school's Associate Professor Peter Timms, the University will work with the Queensland Department of Environment to help stamp out and deter the illegal trade in wildlife.

Besides having a legal obligation to protect the general value of Queensland's wildlife through appropriate land management strategies, the Department also needs the technology and methods to detect illegal wildlife trade activity. (Source: *Australasian Biotechnology*, vol. 8, No. 3, June 1998)

Fish farming for the year 2000

An exciting new research programme in aquaculture is underway that will allow the use of a simple growth factor test to fine tune the environmental and nutritional conditions for fish farming. The project being carried out by the newly formed aquaculture group of the CRC for Tissue Growth and Repair will help fisheries management to maximize their output.

The Centre's newly formed aquaculture group at Flinders University is studying growth factors in farmed fish. A dominant research goal of this group is to determine whether the levels of growth factors in fish are linked to growth or responses to environmental or nutritional stress.

Such are the exciting commercial possibilities of this work that the Fish Research and Development Corporation have provided significant funds for the work. GroPep, the Centre's commercial agent, is conducting parallel research in this area assisted by a Graduate Based Project grant from AusIndustry's R&D START Program.

Aquaculture is a rapidly growing area and currently supplies 16 per cent of Australia's fisheries products. However, one of the major challenges facing those associated with the industry is maintaining the balance between commercial success and sustainability of resources. The work of the CRC research team will be crucial in assisting the fish industry

to achieve this balance by allowing operators to increase production and also measure growth rates of fish under various environmental conditions. The development of such a test would provide a valuable export to Australia and benefit the aquaculture industry worldwide.

Collaborations with scientists at University of Queensland and University of Tasmania further highlight the group's determination to see Australia as a key player in the aquaculture industry. (Source: *Australasian Biotechnology*, Vol. 8, No. 3, June 1998)

Science alliance to boost coverage

Five major science groups announced on 29th March 1998 that they had formed a new coalition to promote the public understanding of science and technology in Australia. The first event the Committee will oversee is a three-day forum in Melbourne from 7-10 May called Science NOW!

The Committee consists of the Presidents (or their representatives) of the following bodies:

- The Australian Academy of Science (President Sir Gustav Nossal)
- The Australian Academy of Technological Sciences and Engineering (President Mr. Tim Besley)
- The Australian Science Communicators (President Professor Ian Lowe)
- The Federation of Australian Scientific and Technological Societies (President Professor Peter Cullen)
- Australian and New Zealand Association for the Advancement of Science (President Professor Paul Adam)
- The National Press Club (President Mr. Ken Randall)
- Dr. Jim Peacock, a member of both Science Academies and head of CSIRO's Division of Plant Industry, has been elected as Chair of the Committee. He said that the cooperation of these peak science councils was a major step in boosting the public communication of science and technology in Australia.
- The forum was initially suggested by Australian Science Communicators, and builds on the experience of the ANZAAS Congresses. Dr. Peacock said that the first two forums are to be held in Melbourne with the support of the Victorian Government and assisted by the Commonwealth Department of Industry, Science and Tourism. The Committee expects to be inviting the other States to bid for the right to hold this showcase for science and technology in future years.

(Source: *Australasian Biotechnology*, Vol. 8, No.3, June 1998)

Government's new initiative on biotechnology: Biotechnology Task Force

The Department of Industry, Science and Tourism will establish a Biotechnology Task Force to advise the Government on strategies for development of the Australian biotechnology sectors.

To ensure that all relevant factors are taken into consideration, the Task Force will consult widely with other Commonwealth Departments and State Governments, industry, research institutions, consumer groups and other stakeholders. The Task Force will take into consideration other Government programmes and initiatives and report on:

- Opportunities for the development of internationally competitive biotechnology-based economic activities;
- Impediments to the development of such activities;
- Strategies to address issues such as:
  - Technology development and diffusion
  - Intellectual property management and ownership
  - Access to venture capital
  - Market access and promotion
  - Regulation, biosafety and biodiversity
  - Public awareness
  - International agreements on intellectual property and other issues.

Two initial projects which the Biotechnology Task Force will support are:

- Preparation of an Australian Biotechnology Director, in collaboration with the Australian Biotechnology Association, to establish the size, scope and capabilities of the industry as a resource and marketing tool;
- International collaboration through support for an Australia-California Biotechnology Partnering Meeting in San Diego, USA, in May 1999.

For information about the Task Force, contact:
Dr. Joe Hlubucek, General Manager, Biotechnology Task Force, Department of industry, Sciences and Tourism. Tel.: (02) 6213 6367; Fax: (02) 6213 6365; e-mail: biotaskforce@dist.gov.au

The Government also released a new biotech brochure in early September 1998 outlining its initiatives and programmes in the biotechnology area. These include:

- R&D start
- Concessional loans for the commercialization of technological innovation
- R&D tax concession
- Pharmaceutical Industry Investment Programme
- Gene Technology Office
- CRC Programme
- Innovation Investment Fund Programme

(Source, *Australasian Biotechnology*, Vol. 8, No. 5, October 1998)

CSIRO to start July trials on atrazine

Trials on a bacteria that may be able to treat groundwater contaminated with the pesticide atrazine will be started in July by CSIRO, the Australian research institute. The trials will be carried out in Perth where contaminated groundwater has caused plant damage in gardens, but the knowledge and technology gained from the project could be applied across Australia, according to CSIRO.

Atrazine, which is commonly used to control weeds in forestry, horticulture and the grain and cotton industries, breaks down when on the surface. However, when 15 metres below the surface and immersed in water that lacks oxygen, the pesticide shows little sign of natural degradation.

While searching for a solution, CSIRO identified a microscopic bug discovered by an Israeli scientist, Raphi Mandelbaum, in a contaminated site in the US. The bacteria, *Pseudomonas citronellolis* (strain ADP), was found to convert atrazine to carbon dioxide.

CSIRO now plans to inject the bacteria into the leading edge of a plume in Perth to check its effectiveness in a real-life situation. (Source: *European Chemical News*, 29 June-5 July 1998)

Trees are the next target of genetic engineers

International demand for wood has grown 36 per cent in the past 25 years and is now a $400 billion business, according to a report on the world's forests published by the Food and Agriculture Organization of the United Nations last year. This puts pressure on commercial tree plantations; and

there are fewer virgin forests left to cut. Hence a new enthusiasm for manipulating the genetics of trees, especially of commercially valuable species.

Given the large number of tree genes and the little that is known about them, tree engineers are starting where other gene wizards have started before them: with a search for genetic "markers". The first step is to isolate DNA from trees with desirable properties such as insect resistance. The next step is to find stretches of DNA—not necessarily in the genes themselves, since this is such a time-consuming process, but in surrogates—that show the presence of a particular gene. Then, when you mate two trees with different desirable properties, it is simple to check which offspring contain them all by looking for the genetic markers.

One firm putting this to use is ForBio, based in Brisbane, Australia. So far, its scientists have identified 600 genetic markers in ten species of eucalyptus, acacia and melaleuca, a temperate tree prized for its oils. The company then breeds trees together to combine such things as salt tolerance and wood quality in a single plant. ForBio hopes to have 10 million of its enhanced trees growing around the world within two years; it already has fast-growing eucalyptus in Indonesian plantations and hopes to get approval to plant its first crop of salt-tolerant trees in Australia's Murray Darling Basin, once rich agricultural land that is "salting up" due to a rising water table. (Source: *The Economist*, 25 July 1998)

Bangladesh

Grameen Bank cancels deal with Monsanto

The Grameen Bank's announcement that it would accept US$ 150,000 from Monsanto (St. Louis, US) to launch the Grameen Monsanto Center for Environment-Friendly Technologies stirred a storm of controversy throughout agricultural and rural organizations around the Third World. The surprise move was unveiled jointly by Muhammad Yunus, Managing Director of the Grameen Bank and Robert Shapiro, Monsanto's Chair and CEO. After widespread international opposition to the deal by such activists as Vandana Shiva and Rural Advancement Foundation International (RAFI), Yunus withdrew from the agreement.

The company's initial grant was to be for loans to Bangladeshi farmers to buy agricultural and rural technologies including Monsanto's own proprietary herbicides, hybrid rice, hybrid maize and cotton seeds. Hybrid rice and maize are biologically incapable of breeding "true" in the second generation. Seeds are either sterile or produce often unwelcome genetic "throwbacks". Although some scientists regard hybrids as a boon to crop yields, there is a growing opinion that the real advantage is that farmers are forced back to the market every year to buy new seeds.

Traditionally, Bangladeshi rice farmers—among the poorest of the poor—not only save seed for replanting, but women breed diverse seed types in order to have varieties suited to their immediate ecosystems and economies. Hybrid seeds could more than quadruple seed costs as well as end forever the process of poor farmers adapting plants to their resource-poor soils.

While Monsanto has stated that it would not provide transgenic seed because Bangladesh does not have a regulatory framework for the approval of genetically-modified organisms, the Grameen/Monsanto announcement was expected to put political pressure on the government to adopt biosafety rules amenable to Monsanto's extensive line of herbicide-tolerant crops.

Since its founding in 1983, the Grameen Bank has pioneered the concept of "micro-credit" whereby the poor—very often women—obtain small loans (often less than $100) without collateral. Muhammad Yunus, the Bank's founder, has shown that the poorest of the poor will repay their debts 98 per cent of the time—a rate far superior to the record of commercial banks either in the South or in industrialized countries. Today, about eight million families obtain micro-credit to launch tiny but profitable ventures such as loans to buy chickens in order to sell eggs. Almost half of the micro-credit activity continues to centre around the Grameen Bank in Bangladesh.

In late July, the BBC reported that Yunus had cancelled the agreement; however, Monsanto has stated that it will move ahead with plans in Bangladesh despite Grameen's withdrawal.

For further information, contact: RAFI, 110 Osborne St., Suite 202, Winnipeg MB R3L 1Y5, Canada. Tel.: (204) 453-5259; Fax (204) 925-8034; e-mail: rafi@rafi.org

RAI-USA, P.O. Box 640, Pittsboro NC 27312 US. Tel.: (919) 542-1396; Fax (919) 542-0069; e-mail hope@rafi.org (Source: *Global Pesticide Campaigner*, September 1998)

Brazil

"Milestone" decision breaks ban on GM crops

Brazil has broken its historic ban on genetically modified (GM) crops by approving Monsanto's *Roundup Ready* soybean seeds. Brazil's National Commission for Biological Security (CTN-Bio) approved the seeds, opening up the world's second-largest soybean producer to the modified crop market.

Luis Antonio Abramides Do Val, director of regulatory affairs for Monsanto in Brazil, called the approval a "milestone". The firm first applied for approval in 1994, and has spent $250 million in Brazil on seeding and herbicide plants over the last three years. Abramides Do Val also hopes pending applications to commercialize the firm's corn and cotton could be approved by 2000.

According to Reuters, once the company plants the beans it will immediately control 2-4 per cent of Brazil's soybean market, and it expects this to rise to 20 per cent within three years. Monsanto claims it now controls 28 per cent of soybean planted land in the US. After two years in Argentina it controls 18 per cent of these crops.

However, the Commission said no decision has yet been taken on labelling the soybeans and their derivatives. In addition, the approval does not yet overturn a recent court injunction which prevents planting of the soybean for the time being. Monsanto said it hopes to overturn the ruling. (Source: *European Chemical News*, 5-11 October 1998)

Brazil wants cut of its biological bounty

A debate is brewing in the Brazilian Senate over legislation designed to ensure that Brazil's citizens share in any profits from crops or medicines derived from the biological wealth of the Amazon and other species-rich regions. Brazilian officials say they hope the legislation will encourage bioprospecting. "We want to establish rules to stimulate the use of biodiversity, not restrict it", says molecular biologist Luiz Antonio Barreto de Castro, an official in Brazil's Science Ministry. But some scientists, while applauding the legislation's goals, warn that it could imperil field research in Brazil. The legislation "is potentially a real

roadblock ... to scientific progress", says Smithsonian Institution biologist Thomas Lovejoy.

The legislation, observers say, has its origins in the collapse of Brazil's rubber industry in the early 1900s after Brazilian seeds were transplanted to south-east Asia and used to start the region's booming rubber plantations. In several other instances since then, foreign organizations have claimed breeding or patent rights to Amazonian plants that might be useful as crops or medicines, such as the pinto peanut.

The first attempt to reverse this trend and formally assert Brazil's ownership of native plants and animals came three years ago. A Brazilian senator from the Amazon region, Marina Silva, introduced a bill that would recognize local citizens' ownership of native species and mandate that any benefits derived from commercial uses of these resources be shared with local tribes. After a series of hearings, a more detailed version of that bill was introduced last year outlining a series of bureaucratic hurdles that anyone who wants to collect and use biological specimens in Brazil must clear.

Supporters had hoped this second bill would breeze through the Senate's education commission before heading for debate in Brazil's Chamber of Deputies. But it has encountered opposition.

Now Brazil's executive branch plans to offer alternative legislation in the next couple of months that would leave it to regulators to devise how to implement the bill's provisions. One issue that must be clarified, says de Castro, is how to ensure that local residents are rewarded for providing knowledge used to identify potentially valuable species. Both the Senate bill and the government's draft version state that folklore has unspecified value—opening the door for local residents to receive compensation and have a say in what happens to their resources, de Castro says. But exactly how to do that is still a debated issue.

Biotech companies hoping to work in Brazil are watching with interest. If Brazil manages to lay out a balanced legal framework that empowers indigenous peoples but does not cut too deeply into a company's bottom line, it could stimulate bioprospecting, says Steven King, a botanist with Shaman Pharmaceuticals in South San Francisco.

But some biologists who collect specimens for research remain wary. "I want these countries to realize the proper return [on their biodiversity]", says Lovejoy. However, he adds, during recent hearings in the Brazilian Senate, research activities were lumped with commercial and amateur collecting. That might lead to unduly harsh restrictions on research, says Lovejoy, who acknowledges that Brazil faces a difficult balancing act: juggling the concerns of scientists with a desire to redress old wrongs and the need to return benefits to its peoples. (Source: *Science*, Vol. 279, 6 March 1998)

Cambodia

Refugee crops return

Traditional varieties of rice that disappeared from the fields of Cambodia during the rule of the Khmer Rouge are making a comeback after samples were discovered in an international gene bank.

Gene banks in Cambodia were reduced to rubble during the fighting of the early 1970s and by the Khmer Rouge's destruction of the country's scientific infrastructure after it took over in 1975. Amid the turmoil, starving peasants often had little choice but to consume their own reserves of seeds.

As their country descended into chaos, Cambodian scientists sent samples from national seed banks for safe keeping in the cold storage facilities at the International Rice Research Institute in Los Banos, the Philippines. Three varieties found there have now been returned to Cambodia.

A new generation of Cambodian plant breeders is rediscovering the unique properties of the lost varieties. Cambodia's rain-fed lowland agricultural environment is highly diverse and requires a range of rice varieties with different growth rates and sunlight sensitivities. The reintroduced species are already producing higher yields than modern varieties. (Source: *New Scientist*, 10 October 1998)

Canada

Toronto gets genomics research facility at children's hospital

The hospital for Sick Children in Toronto has opened up a one-stop genetics research shop with its Center for Applied Genomics.

The 6,000 square foot centre combines the previously separate gene mapping, gene identification, DNA sequencing and DNA synthesis facilities.

"By consolidating everything into one centre we've established a critical mass of equipment, information, and scientists", said the centre's co-director, Steve Scherer, "that allows us to interact and bring things together in a much faster way".

The centre will take on research projects on any genetic matter, but it makes a specialty of chromosome seven. The Hospital for Sick Children has concentrated on the chromosome since a hospital research team working with the University of Michigan located the cystic fibrosis gene there in 1989. The centre acts as a resource of chromosome seven research to scientists around the world. For instance it just supplied a list of candidate genes on the chromosome to a Japanese research team looking into a metabolic disease that only affects Japanese.

With its expanded resources, the centre is concentrating on researching genes on complex diseases such as diabetes, epilepsy and autism. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 17 August 1998)

New rules on human subjects

After four years, Canadian researchers are about to get a new code of conduct for research involving human subjects.

The new guidelines will be issued by Canada's three government research funding agencies—the Medical Research Council (MRC), Natural Sciences and Engineering Research Council, and Social Sciences and Humanities Research Council (SSHRC). The exercise revealed deep divisions within the scientific community over the best way to ensure ethical conduct, with biomedical researchers generally pushing for strict guidelines and social scientists arguing for a more flexible standard. The document represents a delicate compromise on issues ranging from the use of deception in social science research to definitions of what constitutes minimal risk for research subjects.

The tricouncil ethics exercise, unpopular with most academics, was conceived to preempt the government from moving ahead with legislation on aspects of research involving humans. It followed a 1994 report by the Royal Commission on New Reproductive Technologies that recommended legislation to govern scientific activities in this highly charged field, as well as a study that found enormous variance

in the workings of the institutional Research Ethics Boards (REBs) that monitor human experimentation.

For the most part, the guidelines lay out procedures that universities should follow for approving and monitoring such research. But they include a few absolute prohibitions.

To foster compliance, the guidelines attempt to standardize the membership and operations of the estimated 300 to 400 REBs affiliated with universities, hospitals, and research institutes. They also extend the purview of REBs to include a scientific review of all research in the social sciences and humanities.

Although the councils say that the new rules are not a formal code, compliance is required for continued funding. Institutions will, however, be able to tailor procedures on a case-by-case basis, giving full REB review to some protocols and less rigorous, expedited review to those deemed of minimal or "everyday" risk. (Extracted from *Science*, Vol. 280, 5 June 1998).

China

Government approves plans for Pioneer Hi-Bred research centre at Tieling City

The Chinese Government has approved plans to establish a Pioneer Hi-Bred International, Inc. research centre at Tieling City in Liaoning Province. The Centre's efforts will be focused on the development of corn hybrids adapted to growing conditions in the People's Republic of China. Almost all of the fifty million acres in the PRC that are devoted to corn are planted with hybrid seed corn.

The 25-acre Tieling research centre will join Pioneer's network of more than one hundred locations, including those in Thailand, India, Indonesia, and the Philippines. Each contributes data and genetic material for the development of new products for the world market.

The announcement followed the opening of a Pioneer business office in Beijing last January. That office will work with Chinese officials in an effort to set up governmental seed performance testing and establish relationships for marketing Pioneer products. The PRC recently passed legislation giving protection to proprietary genetic material. That law has encouraged Pioneer Hi-Bred officials to feel confident that both the corporation and Chinese farmers will benefit from new seed products. For further information, contact: Richard McConnell, Pioneer Hi-Bred International, Inc., Research and Product Development, 7300 NW 62nd Avenue, P.O. BOX 1004, Johnston, IA 50131 USA. Tel.: +1-515-270-3363; Fax: +1-515-253-2478; e-mail: mcconelr@phibred.com (Extracted from *Diversity*, Vol. 14, Nos. 1 and 2, 1998)

Few geneticists have qualms about endorsing eugenic practices

Chinese geneticists overwhelmingly support the use of eugenics to improve the health of their nation. A survey of 255 geneticists throughout China has shown that most of them favour genetic testing for purposes that their Western colleagues would strongly disapprove of.

Routine genetic testing of job applicants by employers, a practice widely denounced as unethical in the West, was supported by 86 per cent of those polled. The same proportion believed that governments should require premarital tests to detect carriers of hereditary diseases. And 91 per cent believed that couples who carry the same disease-causing genetic mutation should not be allowed to have children.

The survey, conducted by Xin Mao of the West China University of Medical Sciences in Chengdu, also found widespread support for genetic testing of children to see if they are susceptible to diseases later in life such as alcoholism, with 69 per cent in favour.

Mao conducted his survey in 1993, a year before China introduced its controversial Maternal and Infant Health Care Law. This makes premarital checkups compulsory, and allows doctors to order termination of foetuses with "a defect of a serious nature" such as an incapacitating physical disease. The law will also cover mental illnesses such as schizophrenia if suitable genetic tests become available. (Extracted from *New Scientist*, 24 October 1998)

China gets gene development facility

Shanghai New Huang Pu Group Co., Ltd. and Fudan University have established China's first gene development facility, Shanghai Biorigin Gene Development Co., Ltd., with 100 million yuan (US$ 12 million) in capital.

New Huang Pu invested 70 million yuan, as well as 15 million yuan for the venture's imported instruments and reagents, while the university used its research results as its investment.

Shanghai Biorigin will develop clonal genes for medicinals and promote industrial and agricultural production. The company will use 10 per cent of its annual profits to fund its research efforts, which currently includes the discovery of 15 full-length complementary DNA samples per day. By the end of 1999, Shanghai Biorigin plans to set up nine additional laboratories to find 100,000 clonal genes. (Source: *McGraw Hill's Biotechnology Newswatch*, 21 September 1998)

Costa Rica

New Costa Rican law embraces community rights

In May 1998 the new Biodiversity Law of Costa Rica was approved after a ground-breaking participatory legislative process. The approval was a success for civil society organizations, which played a leading role in a matter usually decided by "expert panels" behind closed doors. An amazing 98 per cent of the law was written by a special mixed legislative subcommission made up of representatives of government, universities, business, and grassroots organizations—peasants, indigenous peoples, environmentalists—and the National Biodiversity Institute (INBio).

Among the major achievements of the representatives of civil society was managing to include the following in the law:

- Three permanent seats (one each for indigenous peoples, peasants and environmentalists) in the new National Commission for Biodiversity Management, which will be in charge of developing the major biodiversity-related policies, including access regulations and the national biodiversity strategy. The powerful INBio was left out of the National Commission;
- Prior informed consent (PIC) from local representatives is to be a condition for access to genetic resources, whether in protected areas, indigenous peoples' lands, or private property;
- The incorporation of *cultural objection* as a way to limit or deny access petitions; such objections may be sustained on cultural, spiritual, social and economic grounds;
- Recognition of community intellectual rights, to be formulated in a participatory process by local communities and indigenous peoples during the 18th months following the approval of the law;

- Limiting IPRs on life forms, since intellectual and industrial property rights must now be in accordance with the new biodiversity legislation.

The grassroots organizations—with the continued support of Programa CAMBIOS, of the Costa Rican Universidad Nacional—will now be working on a strategy to influence the implementation process for the new legislation. These organizations are also committed to monitoring developments concerning biodiversity-related treaties and conventions. The national network will lead the work to set up the new community intellectual rights, and will move ahead at the regional level (Central America and Caribbean) on biodiversity resources-related issues.
(For further information, contact: Programa CAMBIOS, Escuela de Ciencias Ambientales, Universidad Nacional, Apartado 86-300 Heredia, Costa Rica. E-mail: silviar@una.ac.cr (Source: *Seedling*, June 1998)

Ecuador

Mangroves are at the mercy of shrimp farmers

After more than two decades of rapid growth, shrimp farms produce about a quarter of the three million tonnes of shrimps that are consumed worldwide each year. Environmentalists say that the rapid expansion of the industry is doing untold damage—particularly to mangroves. These coastal forests are important nurseries for many species of fish, and protect the low-lying land behind them from stormy seas.

Often mangroves are felled to make way for shrimp ponds, many of which are abandoned after a few years. In much of Asia, shrimp farmers find it easier to build new ponds than maintain old ones.

Shrimp farms in India and other Asian Countries tend to be small, so farmers rear shrimp at very high densities to maximize their profits. The ponds generate lots of waste, and farmers dose them with antibiotics to stop outbreaks of infectious disease.

Jason Clay of the World Wildlife Fund (WWF) in Washington, D. C. estimates that farmed shrimp eat less than 30 per cent of the food given to them. The rest of it ends up as effluent that pollutes tidal waters, damaging local fisheries and fouling bays that are not regularly flushed by the ocean.

The source of shrimp larvae for aquaculture is also causing concern. Some larvae come from hatcheries, but the remainder are caught in coastal waters using fine-meshed nets. The nets inevitably trap large quantities of other invertebrates and fish—amounting to 20 or more times the weight of the shrimp larvae.

In Ecuador, about half the larvae come from hatcheries. Rodrigo Laniado Romero, an Ecuadorian shrimp farmer says that he and his follow farmers prefer larvae from this source. His organization, the Ecuadorian Chamber of Aquaculture, has just made a pact with Fundación Natura, an Ecuadorian affiliate of the WWF, to monitor and protect mangroves threatened by shrimp farms. Under the agreement, shrimp farmers will help to enforce recent laws protecting mangroves. In return, the government will provide incentives for farmers to restore damaged wildlife habitats. (Source: *New Scientist*, 21 February 1998)

European Union

European News

The European Parliament has finally approved the Biotechnology Patenting Directive without amendments, thus clearing the way for the legislation to be signed by the Council of Ministers without any further discussion. After more than 10 years of debate, the directive allows for the patenting of biotechnology inventions and was widely acknowledged as being crucially important for the development of the European biotechnology industry.

The European Commission has selected the projects to be funded under the 4th and final funding round of the European Biotechnology Programme. There were 572 proposals to the programme and 154 projects have been selected for funding. Scientific areas that were prominent were: cell factories, neurosciences, immunology and transdisease vaccinology, as well as structural biology. Examples of such projects that will receive funding include the development of gene therapies for neuro-degenerative diseases. Industrial participation in the programme continued to increase with 65 per cent of all applicant consortia containing at least one industry partner.

Overall, the Biotechnology Programme has supported 456 projects and represents an investment of Ecu 533 million. The next major opportunity for EU-funded biotechnology research will now occur in the future European Science R&D Programme (Framework 5) which is due to start in 1999. Further details of the European Biotechnology Programme are available from: Mr. Stephane Hogan, European Commission, Biotechnology Unit, DGXII-E/1, 200 Rue de la Loi, B-1049 Brussels, Belgium. e-mail: Stephane.hogan@dg12.cec.be

The European Commission is to provide Ecu 426,000 in launching a new initiative, the European Plant Biotechnology Network (EPBN). The network, established in conjunction with pan European industry and academic support aims to (i) promote technology transfer from European plant biotechnology research to European industry; (ii) increase the interactions between researchers and end users of research results; and (iii) inform society of the benefits of plant biotechnology. Overall, EPBN aims to maximize the benefits gained by EU supported plant biotechnology research involving 394 laboratories in 20 countries with a total research investment of Ecu 150 million. The network will be coordinated by a company called AMICA Science EEIG, the Plant Industrial Platform and finally members of the European plant science community. Further details from: Dr. Andy Beadle, AMICA Science EEIG. Tel.: + 44 1603 452 57; e-mail:beadle@bbsrc.ac.uk

A permanent inventory of BIOtechnology REsearch Projects (BIOREP) in the European Union has been created and put on the Internet. It covers ongoing and recently completed biotechnology research projects and contains details of nearly 8,000 individual entries. It aims to further scientific contacts, identify trends in funding and also to generally assist in transnational planning. The BIOREP web site can be found on: http://www.niwi.knaw.nl/cgi-bin/nph-biorep_search.pl

Science-free GM food tests advance

A European Council regulation stipulating that any food product made from some maize and soya varieties containing "foreign" DNA or proteins must be labelled accordingly (*Nature Biotechnology* 16:605, 1998) comes into force in September 1998. In effect, the regulation means that all soya and corn from the United States, and possibly all prepared foods containing them, will have to be tested, creating what some regard as a substantial new and unscientific barrier to trade. There are, however, no officially approved tests on the market that would allow food companies to test for molecules

from genetically modified organisms in their ingredients, no standards protocols outlined, and the lower limit for testing has yet to be defined.

The Confederation of Food and Drink Industries of the European Union (CIAA; Brussels, Belgium) has urged the European Commission (Brussels, Belgium) to establish official methods of analysing novel foods. These methods are "still under discussion".

Under the new regulation, to avoid labelling, tests must demonstrate the absence of both "foreign" protein and DNA in the food. As a result, biotechnology companies are hoping to develop and market assays and suitable testing kits.

In establishing the new regulation, the European Council encouraged the European Commission to study the feasibility of setting a lower limit for the presence of DNA or protein resulting from genetic modification. There is currently no lower limit for contamination—detection of the smallest amount of "foreign" DNA or protein triggers the need for labelling. But this does not take into account the problem of accidental contamination, perhaps resulting from crop cross-fertilization, or from mixing in transportation or manufacturing. The scientific and industrial communities are discussing a threshold of 1-3 per cent, which is based on the GATT rules for the contamination of durum wheat by soft wheat. No deadline has been set for a decision.

The Food and Drug/Consumer Protection Unit of the European Commission's Joint Research Centre (JCR; Ispra, Italy) has validated a qualitative assay (originally developed by German and Swiss scientists) with more than 20 academic, private, and governmental control laboratories from a total of 13 countries. The detection method is based on PCR that amplifies the cauliflower mosaic virus 35S-promotor or the NOS-terminator, either of which is currently present in all genetically modified crops on the European market and in 26 of the 28 crops approved or under approval worldwide. The widespread use and utility of this test makes it at least a candidate as a standard test.

Another JCR, in Geel (Belgium), has developed reference probes of maize and soya flour spiked with 0.1, 0.2 and 0.5 per cent GMO. These probes are now commercially available from the Swiss company Fluka (Buchs) and the JCR so that test developers and food companies can calibrate their tests.

US exporters of genetically manipulated crops are likely to be highly critical of the new labelling regulation. The US Food and Drug Administration (FDA; Rockville, MD) requires food labelling according to the US Food, Drug, and Cosmetics Acts. This stipulates safety as the reason for labelling, rather than the mere presence of molecules from GMOs as in the EU (and soon, Japan). Thus, the FDA argues that there is no reason to label products from genetically modified crops because there is no health or nutritional difference between the modified crops brought to market so far and their established precursors. (Extracted from *Nature Biotechnology*, Vol. 16, August1998)

EC passes new GMO screening

The European Commission's Joint Research Centre (JRC) at Ispra, Italy, has validated a screening method that allows the detection of genetically modified agricultural products (GMOs).

The technique, while useful, does not, however, allow the detection of GMOs in processed foods, working only on intact crops.

The technique, developed by Swiss and German scientists, uses polymerase chain reaction to detect the end groups on the piece of genetic material that has been inserted in the crop to improve its properties. These are known as the 35S-promotor and the NOS-terminator.

To date the test has been trialled on flour made from soya beans and maize, but the Commission says it should be possible to detect at least 26 out of the 28 GMOs currently approved or under consideration, as they all use the same promotor/terminator groups. Tests show that a GMO content of just 2 per cent can be detected reliably.

The advantage of the test is that knowledge of, or a sequence from, the inserted gene is not required for the test. This is essential, says the Commission, "since this makes it possible to carry out controls without being dependent on the information given by producers".

The Commission says it intends to pursue the programme to improve methods of identification and quantification of GMOs.

The European Commission has awarded funds of Ecu 138 million ($153 million) over two years to support 154 research projects in biotechnology across Europe.

Development of new vaccines and therapeutic drugs, optimization of bioprocesses to ensure food safety and environment restoration feature prominently on the list.

Industrial participation in the projects is around 16 per cent, while the proportion of projects with at least one industrial partner is now 65 per cent. (Source: *European Chemical News*, 15-21 June 1998)

First commercial European maize plantings

For the first time in Europe, the commercial planting of genetically engineered maize varieties began in April in Germany. Novartis Seeds' (Basel, Switzerland) BT176 variety contains a triplet of genes encoding *Bacillus thuringiensis*(*Bt*) toxin, resistance to the Basta herbicide, and, controversially, resistance to the antibiotic kanamycin. However, only the *Bt* gene is expressed. The product was approved for import into the European Union in December 1996. Although registration for commercial planting in France and Spain was granted early this year, Novartis has not applied for registration in Germany.

However, the Federal Variety Office (Bundessortenamt, Hannover) allows "premarketing" of 10 tonnes of the recombinant crop to a limited number of wholesalers. According to Rainer Linneweber, spokesperson for Novartis Seeds (Bad Salzuflen, Germany), one tonne of transgenic Bt-maize seeds has been delivered to farmers in Germany and has probably been sown. This quantity would seed just a fraction (350 hectares) of the 1.6 million hectares of maize grown in Germany. Farmers are not obliged to mark fields of BT176 nor to disclose sites in publicly available documents. (Source: *Nature Biotechnology*, Vol.16, June 1998)

Finland

Finns fear and favour biotech

Seventy-one per cent of Finns believe biotechnology will improve standards of living within 20 years, according to a survey of the Finnish public's opinions on science. Commissioned by Finnish Bioindustries (Helsinki, Finland), an association representing pharmaceutical and other companies, the survey asked the public whether biotechnology and other areas of science are useful or risky. When presented

with the undefined term "genetechnology", 58 per cent of Finns considered it useful for pharmaceutical and vaccine development, particularly in gene testing to identify inherited diseases, but almost a third thought it would worsen quality of life over the next 20 years. Seen even less favourably was agricultural biotechnology: around half the population sees genetically modified foodstuffs as risky, and 93 per cent would like genetically modified food products to carry labels identifying the technology used during production. (Source: *Nature Biotechnology*, Vol. 16, November 1998)

France

EU attacks French crop ban

The European Commission has officially warned the French Government to lift a moratorium on the planting of transgenic crops other than corn. The Commission's action represents a toughening of policy against European Union (EU) countries that defy its rulings approving transgenic crops.

France's moratorium has been particularly troubling for the Hoechst-Schering joint venture, AgrEvo. All EU members approved AgrEvo's transgenic canola in June 1997, but the French move has also delayed canola planting throughout the EU for the past 18 months.

France's highest court, the Council of State, has also suspended the planting of Novartis's *Bacillus thuringiensus* transgenic corn until December on the grounds that there were administrative "irregularities" in its approval (Source: *Chemical Week*, 14 October 1998)

Crop circles

France's highest court has ruled that maize genetically engineered to produce the insecticide Bt may not be sold there. The court concluded that the French Government had not properly assessed whether a gene for antibiotic resistance, also added to the maize by the Swiss company Novartis, posed a risk to public health. (Source: *New Scientist*, 3 October 1998)

Genetic crops

The French Government announced that it will finally approve the sale of two varieties of genetically modified maize, allowing the crop to be imported across Europe. But, France has also imposed a moratorium on the use of other modified crops.

The approval for the maize varieties—developed by Monsanto and AgrEvo of Germany—will avert a threatened trade war between the US and Europe. Under European Union law, the maize must be approved by France before it can be imported into other member States. France's reluctance blocked US maize exports to Spain and Portugal. US economists claim that over half of 1998's exports were lost as a result.

US Vice President Al Gore, who had personally appealed to French Prime Minister Lionel Jospin to approve the maize, said the decision was good news for the US economy and US farmers. "The evidence is overwhelming—genetically modified foods can provide us with an abundant, affordable, nutritious and safe food supply", Gore said in a statement. "I am glad that France has recognized what we here in the US have long known."

The French Prime Minister announced a two-year moratorium on sales of genetically modified rapeseed and other crops which pose a high risk of cross-fertilization with other plants. This includes AgrEvo's herbicide-resistant rapeseed. (Source: *Chemistry & Industry*, 17 August 1998)

Citizens' conference says "yes" to second generation GMOs

French citizens have given a carefully considered vote of confidence to genetically modified plants and crop products following a two-day "Conférence de Citoyens". This result could have a profound impact on the future of genetically modified organisms (GMOs) in the European Union.

The 14-member citizen's panel—selected by an independent polling organization as a representative selection of the French population—analysed the impact of GMOs on health, the economy and the environment.

Although the outcome of the debate is not binding, France's Government has said it will take the panel's views into account.

The panel acknowledged that GMO technology offers many benefits, even arguing that some people might prefer foods containing GMOs, but expressed concerns about negative effects. These centred on the potential for unchecked proliferation of GMOs, the fact that some GMOs carry antibiotic-resistant marker genes, and the potential for creating strains resistant to all known herbicides.

Some members pressed for a moratorium on trials until safety measures could be put in place. But the panel as a whole encouraged researchers to work towards "second generation" GMOs designed to minimize risks.

To ensure safety, the panel concluded that independent experts should assess risks and that public sector laboratories should maintain systematic surveillance of GMOs. They also advocated the establishment of a "world bank" of modified gene sequences. These would be made freely available to all researchers, who would have to deposit sequences they create. Some members of the panel also argued for the development of a "super herbicide" capable of killing off all GMO strains. (Source: *Chemistry & Industry*, 6 July 1998)

Germany

Government pledges extra cash to develop genomic research

Germany is pushing ahead its development of biotechnology with the announcement of plans by the Federal Ministry for Science and Technology (BMBF) to widen its financial support for research into human and plant genomic systems.

Announcing additional funding for a human genome research cooperation begun in 1995 and the start-up of the plant genomics project, research minister Jürgen Rüttgers said that pooling the know-how of non-profit research institutions and private companies makes the scheme "unique in the world". He added that the commercialization of findings, which has already begun in the drug sector, could help to make Germany more competitive internationally in plant biotechnology too.

Rüttgers has promised funding of more than DM 100 million ($60 million) in 1999-2001 to phase two of the human genome research collaboration in which eight German pharmaceutical producers are participating.

Since 1995, the ministry has awarded DM 136 million, and the German research society (DFG) DM 8 million to 58 projects. The first stage was devoted to charting genes, but

scientists will now compare human genes with those of model organisms such as the mouse or fruit fly.

Work is expected to get under way in autumn 1999 on the genome analysis in plant biological systems (Gabi) project. Some 12 companies, including agrochemicals producers AgrEvo, BASF and Bayer and nine seeds producers, have founded a commercial association to pursue patents and licensing. Like drugmakers, participants in the plant scheme will also contribute their own funds. The collaboration may be extended to food producers and companies in France.

The first stage will focus on basic research into the genomics of the plant *Arabidopsis*. BASF and the Max-Planck Institute for Molecular Plant Physiology recently announced the creation of a new biotech firm, Metanomics, expressly for this purpose. This project's second phase will try to develop plants that are resistant to disease or high in protein/fatty acids or that are suitable as industrial fuels. (Source: *European Chemical News*, 5-11 October 1998)

India

Genomic deal

India's Department of Biotechnology (DBT; New Delhi) has signed an agreement with France's Centre National de la Recherche Scientifique (CNRS; Paris), to focus on the study of the human X chromosome and the silkworm genome. Mapping the genome of the malaria parasite *Plasmodium vivax* may be added in future, as well as a cross-national study of tissue samples to map epilepsy genes. Intellectual property rights will be jointly owned and resulting revenues will be shared. India entered the agreement in an effort to build up its technological capability for mapping, sequencing, and interpreting genomic sequences, and that DBT chose France because it was "impressed by the openness of French scientists". (Source: *Nature Biotechnology*, Vol. 16, August 1998)

Uranium extraction

Indian scientists are considering using a bacterium to boost the state's controversial nuclear weapons programme.

The State-funded Agharkar Research Institute (ARI) in Pune, which specializes in biotechnology, believes that a sulphur-eating microorganism called *Thiobacillus ferrooxidans* should be used to extract uranium from India's depleted reserves.

Demand for uranium in India is likely to far outstrip supply.

Thiobacillus ferrooxidans is commonly found in waste uranium ore or acidic mine water. Feed it metal sulphides and it will produce the soluble form of uranium from insoluble ore. From then on, isolating the metal is simple.

India currently extracts uranium by the conventional method of concentration and ion exchange. But the reagents required to do this are expensive, and the by-products are pollutants. The method is also impractical where the uranium concentration in ore is low—and much of India's known ore reserves contain only between 0.05 and 0.08 per cent uranium.

Microbial mining, says Arvind Agate, director of ARI can cut costs by half because bacteria provide the reagent, so the method can be used to extract uranium from low-grade ores.

To extract the uranium, low-grade ore is piled in a heap on an impermeable surface and sprayed with a solution containing ferrous iron sulphide mixed with the microbes. *Thiobacillus ferrooxidans* then goes to work generating a ferric sulphate, a reagent that transforms insoluble uranium to its soluble state.

The ferric ions oxidize uranate (insoluble tetravalent uranium) to its soluble hexavalent state. The radioactive metal then dissolves in the acidic solution and can be recovered by concentrating the solution and purifying it by precipitation and ion exchange.

The Atomic Mineral Division of the Department of Atomic Energy is also looking at new uranium deposits. The Bhaba Atomic Research Centre is involved in the extraction process.

Although the Jaduguda-Singhbhum belt of northern India looks to be the most promising source of uranium at present, other mines could be established on the Deccan plateau to the south of the Himalayas, where uranium deposits have been found. (Source: *New Scientist, 11 July 1998)*

Monsanto targets India

Monsanto's new $5 million state-of-the-art R&D centre on the Indian Institute of Science (IIS) campus in Bangalore has been established to "create products for India on a global basis". The centre—the first by a multinational seed company in India—will receive $3 million annually from Monsanto, about 10 per cent of India's total budget on biotechnology. The centre will initially focus on plant biotechnology to breed drought-, insect-, and herbicide-resistant crops (mainly cotton and soybean), but will move on to life sciences, bioinformatics, genomics and nutrition. The centre proposes to tackle two common disorders among Indians—iron and vitamin A deficiency—by enhancing the nutritional quality of commonly eaten plants through genetic engineering. As part of the seven-year agreement for space on the IIS campus, the centre will "provide an opening for our biotechnology graduates", says IIS director Govindarajan Padmanabhan. Monsanto may be aiming to have climate-adapted engineered seeds ready for when India begins patenting agricultural products: As a member of the World Trade Organization (Geneva), India is obliged to change its patent law to allow product patenting; an amended bill is before parliament. (Source: *Nature Biotechnology*, Vol. 16, July 1998)

Japan

Japan gene therapy deal

Fuso Pharmaceutical Industries, an Osaka-based manufacturer of injection and dialysis solutions, and the US venture company GenVec (Rockville, MD) announced a five-year collaboration to develop and commercialize gene therapies for treating human cancer. Fuso will provide R&D funding, receive commercial rights in Japan, as well as having the option to commercialize jointly developed products in the Republic of Korea and Taiwan. GenVec will receive royalties on products commercialized by Fuso, and retain commercial rights throughout the rest of the world. (Source: *Nature Biotechnology*, Vol. 16, June 1998)

Republic of Korea

The biobusiness boom

In line with the Government's drive to boost Korea's bioengineering technology, more venture businesses are moving into the nascent bioindustry.

Using genetic engineering, they are developing new food stuffs and medical and pharmaceutical products. Though

the number of items they are producing is currently limited, these are expected to grow rapidly as their R&D efforts gain pace.

The current focus of companies in this sector is niche markets in the medical and chemical industries. In the near future, however, they will operate in the mainstream markets of many industries.

Many pundits are predicting that the bioindustry will emerge as the main engine of growth, a function now handled by the information technology (IT) industry.

While bioventure businesses began sprouting up in Korea in the mid-1990s, a bioindustry boom began in the US and the UK in the late 1970s, paralleling that of the IT industry.

In the US alone, as many as 1,500 bioventure businesses are now active, employing up to 140,000 personnel. The global bioindustry market, which reached $10 billion in 1992, is forecast to grow to $100 billion in 2000 and $304 billion in 2005, an annual growth rate of 22 per cent.

The domestic market is expected to increase from 325 billion won in 1995 to 1.4 trillion won in 2000, jumping again to 23.5 trillion won in 2005, according to an industry forecast.

Industry experts set Korean firms' standard in basic technologies at around 60 per cent of market leaders in advanced countries.

Their technological expertise in developing new materials is known to be far behind industry leaders, at the 20 per cent level. But despite the current situation, an increasing number of high-class researchers are pouring into the bioventure sector.

What matters most is attracting vital investments from the government or businesses that are interested in the field.

Most recently, Korea's first "bioventure mart" was held in July, and the nation's 10 leading bioventure businesses participated in order to attract necessary funds.

It was the first time bioventure businesses held such an event to promote their projects and attract investment from other venture firms, financial institutions, and businesses.

Most of those who open new businesses in the bioventure sector are professional researchers with experience gained at colleges or research centres affiliated with large conglomerates and other state-funded institutes. Most have a staff of between five to 15 workers.

A year ago, there were no more than 20 companies dedicated to the bioventure industry. But this year, the number had risen to 50 by the end of July. Some have formed a coterie.

Since it often takes some time for bioventure businesses to produce products, researchers are urged to make consistent investments in research and development (R&D). Though it may be some time before these investments see substantial returns, scientists and researchers in the field are heartened by the progress of the past few years and hope that the bioindustry will emerge as a key sector of the 21st century.

Unlike diagnostic kits popularized earlier, Korea's gene therapy industry is expected to remain competitive with those of advanced countries, since its is in the initial stage of development internationally.

Gene therapy is expected to emerge as a leading high-tech industry of the 21st century, with market capacity likely to reach five to six trillion won in 2000.

Another fast-growing overseas sector is biobody industry, which is paving the way for the cultivation of human body parts in the laboratory.

The industry produces materials such as artificial skin, artificial bones and artificial muscles that can serve as functional substitutes.

The international market capacity of the biobody sector, which includes artificial viscera and bones, is estimated at $80 billion (about 96 trillion won) annually. (Extracted from *Newsreview*, 12 September 1998)

Malaysia

New centre for gene analysis in Malaysia

The National University of Malaysia (UKM) has announced the opening of its new Gene Analysis and Technology Centre (Gene Centre) on the grounds of its campus in the township of Bangi, about 30 km from Malaysia's capital, Kuala Lumpur. The Centre was set up to encourage the study of molecular biology and biotechnology for the purpose of both research and teaching. The three main focus areas of the Centre's activities are genetic engineering, DNA technology and protein engineering. The Centre is currently staffed by five academic staff members and has more than 20 postgraduate students, including several from overseas. For more information of the Centre's activities, contact Dr. Zulkeflie Zamrod at zza@pkrisc.cc.ukm.my (Source: *Australasian Biotechnology*, Vol. 8, No. 4, August 1998)

Malaysia plans biotech complex

Malaysia is making plans for a large biotechnology industrial complex. Already, a candidate 60-hectare industrial site has been secured in the province of Negeri Sembilan on the Malaysian Peninsula's south-west side.

The bio park would be finished just after the turn of the century.

According to local reports, a business organization has been set up to manage the project and construct production facilities over a four-year period.

The project's first phase will involve the construction of facilities to produce glucose, maltose, caramel, and citric acid.

All these products are aimed at making effective use of Malaysia's agricultural resources, and exports to neighbouring markets are also anticipated. (Source: *McGraw Hill's Biotechnology Newswatch*, 4 May 1998)

New Zealand

Cloning to the rescue

A disappearing breed of New Zealand cattle has been given a new lease of life, thanks to cloning technology.

In 1992, a cow named Lady and some frozen bull semen were all that was left of the Auckland Island breed. Making Lady pregnant with the sperm proved difficult. So David Wells of the Ruakura Research Centre in Hamilton fused cells from Lady's ovaries with eggs from other cattle which had their own genetic material removed, a cloning technique similar to that used to produced the ewe Dolly.

The first calf was born on 31 July by Caesarean section. Experts say this heralds the use of cloning to save endangered species.

Singapore

Developing countries need to popularize science

In the industrialized West, science and technology are the tools that nations have used to improve their social and economic conditions. Numerous developing nations have

attempted to follow suit by establishing policies that foster science and technology with the aim of bringing about similar improvements in their societies.

While these efforts have led to some spectacular successes, particularly in East Asia, many developing countries have found the route a difficult one. They have initiated policies aimed at industrialization, encouraged foreign investment, promoted technological education, emphasized research and development, and instituted economic reforms. Yet it has not led to the success they seek. Shortages of funds, expertise and know-how can all take some of the blame. But a chief obstacle is that a scientific culture has not taken deep root in the societies of these countries. Their people have a limited understanding of how science and technology can help them and their nations.

In Singapore the authors found that science centres can play a vital part in providing this all-important scientific grounding. These centres have a significant role in hybridizing scientific knowledge with the innate cultures of a developing nation, and catalysing strong socio-economic development.

The idea of science centres is a comparatively new one. Their aim is primarily to familiarize people with science and technology and teach the basic concepts involved by imaginative and enjoyable means—through creative exhibitions, exciting educational programmes, films and more recently through lively websites. They are popular with those who use them, and attract favourable comment from media and educationalists alike. Such science centres have become part of the scientific and educational infrastructure of numerous nations.

Singapore began to recognize the need for such an organization as long ago as 1968. By 1975 the Ministry of Science and Technology was outlining the spirit of the idea in a report, *Science and Technology for 2 Million People*. The Singapore Science Centre (SSC) will, through its exhibits and science education programmes, popularize science and encourage talented people in the schools to study engineering and the skills necessary to maintain a modern industrializing society. The key idea was that the SSC would help to explain science to the general public in such a way that the "Man on Orchard Road" would be able to understand how a skilful and judicious use of technology has raised his standard of living and improved the environment in which he and his family lived.

The SSC opened in 1977, and in its first year it had more than 200,000 visitors. Over the past decade it has attracted close to a million visitors each year. Other science centres have sprouted in Hong Kong, Taiwan, Australia and in India. Yet the idea has not been taken up everywhere. There are surprisingly few science centres in the developing countries of Africa, for example. Those countries would have much to gain by setting up science centres for their people.

The basic requirements for a science centre are a plot of land on which to build it, workshop facilities, and a range of scientists, teachers and administrators to manage the operations. Numerous useful "cookbooks" are now available for setting up exhibits and websites, and for jump-starting science promotional programmes. Local expertise and resources have much to contribute to such an exercise.

In many countries with science centres, the state has been the prime mover. The foundation grant and funds to keep a centre running are but a tiny fraction of the state's annual spending. But the potential dividends it can confer are huge. Perhaps funding could be sought from international agencies such as UNESCO, or from the richer countries as part of their overseas development aid programmes. A goal for the coming millennium would be to set up a global network of science centres. Such a network should be free of geopolitical and other non-scientific influences. Its aim would be to promote the universality of science among all humanity.

Leo Tan Wee Hin is president of the Singapore National Academy of Science and a former director of the Singapore Science Centre.

Ramanathan Subramaniam is a senior scientific officer at the centre.

Further information about the Singapore Science Centre is available at http://www.sci-ctr.edu.sg (Source: *New Scientist*, 20 June 1998)

Switzerland

Swiss reject gene ban

On 7 June 1998, Swiss voters overwhelmingly rejected a proposal for a moratorium on the cultivation of genetically modified crops and bans on research on transgenic animals and the patenting of genetically modified organisms. Although the turnout was low almost 1,250,326 voted against the ban, whereas only 625,227 voters favoured it. Pre-election polls had suggested the referendum was too close to call, but it appears many of the "undecided" ultimately decided to reject the ban because of possible repercussions for employment and the economy. The Swiss Working Group on Genetic Engineering—a coalition of about 40 environmental, consumer protection, and small farmers' groups—obtained 111,000 signatures last year to bring the proposal to a vote and have vowed to continue their efforts to curb genetic engineering. (Source: *Nature Biotechnology*, Vol. 16, July 1998)

United Kingdom of Great Britain and Northern Ireland

Diagnostics partnership

One of the UK's leading science institutions has teamed up with a biotechnology firm to develop a medical technology that promises to be literally breathtaking.

Boditech Diagnostics—a 49/51 joint venture between London University's Imperial College and Kiotech, a London-based firm specializing in odour-related biotechnology—will develop instruments which use breath and body odour to identify and monitor a range of medical conditions.

The ultimate goal of the company is to produce domestic "breathalysers' that would allow people to monitor medical conditions at home. These devices would not require blood or tissue samples, and could provide a reliable result almost instantly.

An Imperial College spokesman says that the devices could, in principle, identify any disease that causes distinctive volatile organic chemicals to be emitted in the patient's breath or through the skin. Research will initially concentrate on respiratory infections, but liver and kidney diseases and diabetes will also be early targets. Eventually, such devices may even be able to diagnose such seemingly improbable conditions as schizophrenia.

Boditech plans to put a prototype device into clinical trials in 2000. Initial tests will involve doctors using the instrument to determine which antibiotics are suitable for patients with colds or sore throats. The first commercial devices could reach doctors by 2002, with domestic models

available several years later. (Extracted from *Chemistry and Industry*, 21 September 1998)

Organics must abide GMOs

A UK High Court ruling has permitted a trial of genetically modified (GM) maize in Devon, despite its proximity to an organic farm producing sweetcorn. The farm owner had concerns that if his crop were cross-pollinated with GM maize it would lose its organic status. However, following evidence from the Advisory Committee on Releases to the Environment (ACRE; London), which advises the UK Government, the judge ruled that the farmer's case was "unarguable". ACRE presented the court with an assessment of the likelihood that the GM maize would cross-pollinate with the organic sweetcorn. Taking a 200 metre separation between the crops, accounting for wind direction and the timing of flowering, ACRE calculated the best and worst case scenarios—no cross-pollination or 1 in 1,000 kernels cross-pollinated, respectively. On average the group estimated that 1 in 40,000 kernels would be cross-pollinated. This falls well within the 99.9 per cent purity of seed required by international standards. In fact the crops will be 2 km apart, 10 times further apart than in the theoretical case ACRE calculated. (Source: *Nature Biotechnology*, Vol. 16, August 1998)

UK group advises GMO halt

The closest advisers to the UK Government on nature conservation in England have called for a three-year moratorium on the release of genetically modified (GM) crops. Government-funded English Nature (London) believes, "modern agricultural practices have already caused significant decline in farmland species and that the introduction of genetically modified crops could increase this pressure". A position statement from the advisory body says English Nature will "continue to recommend a moratorium on genetically modified crops until current research has been completed and evaluated". The group considers the release of GM crops appropriate only when "the use of GMOs in agriculture does not lead to changes in land use and management that are detrimental to wildlife using farmland". However, English Nature also says it is "certainly not against the development of GMOs that could benefit the natural environment". (Source: *Nature Biotechnology*, Vol. 16, August 1998)

Mitsubishi Chemical in research alliance

Mitsubishi Chemical (MC) is making inroads into life-sciences with a seven-year, multimillion pound research alliance with Imperial College (IC), London.

MC will support a new Genetic Therapies Centre which will open at IC's South Kensington campus in the spring of 2000, although a "virtual centre" will be established immediately. The company will also support IC's Process Systems Interdisciplinary Research Centre (IRC), which specializes in advanced general purpose process simulation software for design, planning and scheduling of multi-purpose plants.

Although the research will cover the full range of MC's industrial activities, the majority of the funding will go towards gene therapy. Investment will increase substantially as gene therapy moves into clinical trials, he added. IC has experience in developing gene therapy for cystic fibrosis and future developments will focus on the critical area of vector development. (Source: *European Chemical News*, 18-24 May 1998)

United States of America

New EPA regulations

The US Environmental Protection Agency has made a bold move in announcing that it intends to regulate the release of genetically modified organisms into the environment. Despite the cost (approximatively $10.000 to file) and the delay (at least 90 days) involved with submitting pre-manufacturing notices to the EPA, biotechnology product manufacturers will benefit from the regulations. They should welcome the EPA's recent move, a strictly legal interpretation of the influence it already exercises under the Toxic Substances Control Act and the Federal Insecticide, Fungicide, and Rodenticide Act. (Source: *Nature Biotechnology*, Vol. 16, November 1998)

US standards for fish farm wastes

Biological and chemical wastes from agricultural feedlots and fish farms are a major source of water pollution in the United States, according to the Environmental Defense Fund (EDF). However, current standards for discharges from feedlots are inadequate, and the US Environmental Protection Agency (EPA) has never established national standards for waste water discharges from fish farms—which can release herbicides, antibiotics and other farm chemicals directly to surface waters.

Fish farming, which includes production of saltwater fish, freshwater fish and shellfish, is now the fastest growing sector of US agriculture, according to EDF. Data from the US National Marine Fisheries Service and the Food and Agriculture Organization of the United Nations indicate that US production of farmed fish increased by more than 50 per cent in value over the past decade and is now approaching US$ 1 billion per year. Major species cultivated include channel catfish, rainbow trout, Atlantic salmon and shrimp. These species may be grown in ponds, tanks or cages placed directly in bays and estuaries. Producers range from individual owner-operators to large corporations.

Although some states have established standards, most US fish farms do not currently treat their wastes. However, EPA is now evaluating fish farming as an industrial category for the development of "effluent limitation guidelines" under the Clean Water Act. If developed, these effluent guidelines would establish national standards for discharge of fish farm wastes and could prescribe best management practices for fish farms.

More information on fish farming in the US is available from EDF. Murky Waters: Environmental Effects of Aquaculture in the US can be downloaded from EDF's website: www.edf.org/pubs/Reports/Aquaculture/. Printed copies can be ordered for US$ 20: phone toll free (800) 684-3322; E-mail: members@edf.org (Source: *Global Pesticide Campaigner*, Vol. 8, No. 3, September 1998)

US consortium reaches synthesis "milestone"

A US consortium of four companies and a government institute has announced what it describes as a "a major milestone" in the development of biotechnological routes to chemicals from renewable resources.

The parties involved include Genencor International (a joint venture between Eastman Chemical and Cultor), Eastman Chemical, Electrosynthesis, MicroGenomics and Argonne National Laboratory. The new fermentation process

combines several enzymatic steps within one production organism to synthesize a desired chemical using glucose as the main bulk raw material. Innovative downstream recovery and purification steps, such as electrodialysis, contribute to the overall success of the technology.

The process is "fully scalable", said Robert Clemens, director of industrial biochemical programmes at Eastman Chemical. "The process has been run at scales with which any industrial company would be comfortable."

The first chemical produced using the process is ascorbic acid, which has a worldwide market of about US$ 600 million, although the technology could be applied to a wide range of chemicals. It is estimated that the process could cut the capital cost by one half compared to the conventional Reichstein chemical technology. The partners expect to commercialize the technology in cooperation with another company.

Although Eastman Chemical does not currently have any biotechnology production, the ascorbic acid project is regarded as a good "entry process" into biotechnology, according to Clemens. (Source: *European Chemical News*, 31 August-6 September 1998)

EPA urged to move on Bt

In a consensus statement, the members of a scientific panel—convened in February 1998 by the Environmental Protection Agency (EPA, Washington, D. C.)—now recommend that the agency "require the use of structured refuges" as a way of preserving *Bacillus thuringiensis* (Bt) insecticidal toxins and extending the useful lifetime of crop plants that are genetically engineered to produce these insecticides. Recognizing that the risk of insects developing "strong resistance to Bt toxins is real", the members of the panel say that mitigating those risks is "in the public interest". However, they also say that the EPA needs to provide growers a "sustainable approach" for guarding against Bt resistance and that they should not be discouraged from using "this very valuable and environmentally friendly technology". (Source: *Nature Biotechnology*, Vol. 16, June 1998)

Activists demand the right to know what they are eating

Two pressure groups are suing the US Food and Drug Administration (FDA) in an attempt to force it to make safety testing and clear labelling compulsory for all genetically engineered food.

The lawsuit, filed by the International Center for Technology Assessment and the Alliance for Bio-Integrity, both based in Washington, DC, is also demanding the recall of products containing a total of 36 genetically engineered crops.

US federal law states that food that has undergone a "material change" must be labelled. The FDA has interpreted this to include irradiated vegetables, pasteurized milk, or fruit juice reconstituted from concentrate. Joseph Mendelson, legal director of the International Center for Technology Assessment, argues that genetically engineered food has undergone changes that are at least as significant.

The pressure groups also want safety tests to be carried out on genetically engineered foods, similar to those required for food with new additives. (Source: *New Scientist*, 6 June 1998)

Biotech pipeline numbers 350

A survey from the Pharmaceutical Research and Manufacturers of America (PhRMA) counts 350 biotechnology drugs in development, an increase of 66 drugs since 1996.

In addition, the PhRMA counted 140 pharmaceutical and biotechnology companies involved in testing these products.

The Washington, D. C.-based organization says that the "biotechnology revolution has already given patients 54 state-of-the-art medicines". The first of these was Humulin, a recombinant insulin, approved in 1982.

The PhRMA estimates that about 60 million patients have benefited from these drugs.

PhRMA President Alan F. Holmer said that 19 products were approved by the US Food and Drug Administration in the past two years. These include: a clotting factor for haemophilia B; platelet boosters for patients undergoing chemotherapy; a monoclonal antibody for non-Hodgkin's lymphoma; a humanized monoclonal antibody to prevent rejection of transplanted kidneys; an interferon for hepatitis C, and a thrombolytic for heart attacks.

About half of the biotechnology drugs in development—151—are targeting cancer, with 30 aimed at melanoma alone. Another 29 are aimed at HIV, AIDS and AIDS-related diseases and 10 are targeting autoimmune disorders, including rheumatoid arthritis and lupus. (Source: *McGraw Hill's Biotechnology Newswatch*, 4 May 1998)

D. RESEARCH

Research on human genes

Alzheimer's protein may improve memory

A protein that builds up on neurons in the brains of Alzheimer's patients can boost the memories of mice, a study by scientists in the United States and France has found. They say the results may lead to a better understanding of learning and memory processing, and how they go wrong when Alzheimer's sets in.

Fragments of a protein called amyloid precursor protein (APP) form neural plaques in the brain that are a hallmark of Alzheimer's disease. But these plaques have created a "chicken and egg" problem for scientists, who do not know if they cause memory loss or are a by-product of the disease process.

Steven Paul of Lilly Research Laboratories in Indianapolis, Indiana and Arielle Ungerer at the Louis Pasteur University in Strasbourg were searching for chemicals that might enhance memory when they decided to try APP. They assessed the memory abilities of mice using standard tests.

The researchers injected mice with APP, either after an initial training session or before they had acquired the skill, and found that these mice performed markedly better on subsequent trials than mice that did not receive the protein. In addition, when they injected mice with a drug called scopolamine that causes memory loss, they found that administering APP counteracted the drug's effect.

Paul says this is the first direct evidence linking the normal function of APP to learning and memory. He believes the work may lead to new ways of treating normal age-related memory loss as well as Alzheimer's.

However, speculation about therapeutic applications is premature until researchers learn exactly what the protein does in the brain. (Extracted from *New Scientist*, 24 October 1998)

Blind fate

A gene that is linked to Alzheimer's disease may also be involved in age-related macular degeneration, a type of blindness that afflicts more than 1 in 10 people over the age of 80.

One version of the gene for apolipoprotein E (Apo-E), which helps to maintain nerve cell membranes, substantially raises the risk of Alzheimer's. A team led by Caroline Klaver at Erasmus University in Rotterdam checked the Apo-E gene of 88 patients with vision loss as a result of age-related macular degeneration (AMD), along with that of 901 controls. They found that another version of Apo-E increases the risk of AMD by 50 per cent. (Source: *New Scientist*, 4 July 1998)

Unfolding a cure for Alzheimer's

Researchers have found a way to halt the growth of a substance thought to cause Alzheimer's disease. The team, from New York University School of Medicine, says that the discovery could lead to a new method of fighting the degenerative disease.

One of the hallmarks of Alzheimer's is the build-up in the brain of plaques of a protein called amyloid. Many researchers now believe that it is these plaques that cause the death of nerve cells characteristic of Alzheimer's, particularly in the parts of the brain linked to memory and learning.

The researchers, led by Claudio Soto, designed a fragment of protein, called a peptide, which completely blocked the formation of amyloid plaques in rat brains and destroyed existing amyloids. The peptide also prevented nerve cell death in cultures of human nerve tissue.

Amyloid plaque is deposited in the brain as a protein structure called a β-pleated sheet. The sheets appear to form when the protein folds wrongly. Soto's peptide, which is just five amino acids long, mimics the part of the amyloid protein that regulates folding.

"The β-sheet breaker peptide interferes with the folding process so amyloid plaque cannot form", Soto explains. "The breaker will also dissolve any amyloid that is already formed." No other compound has ever been able to stop amyloid formation in animals, Soto adds.

Although he does not know exactly how the β-sheet breaker works, Soto suggests that it stabilizes amyloid's normal structure, preventing it from folding into a β-sheet. Alternately, the peptide may insert itself into the plaque and halt its growth.

The peptide may also be useful in treating other diseases caused by defective protein folding, Soto Says. (Source: *Chemical & Industry*, 6 July 1998)

Mutation for most common form of Alzheimer's may lead to treatment

A newly found genetic mutation linked to late-onset Alzheimer's disease may shed light on the protein pathways of the disorder and lead to new drug therapies or preventatives, according to a new study.

Researchers at Massachusetts General Hospital in Boston and their colleagues also found that a protein associated with the mutated gene, known as alpha-2 macroglobulin (A2M), interacts with proteins associated with other Alzheimer's-related genes.

The findings may lead to a better understanding of the protein processes that lead to Alzheimer's, and possibly to new drugs to prevent or treat the disease, the researchers said.

A2M has been known as a protease inhibitor, meaning it controls the activity of enzymes that break down other proteins, but the researchers linked it to Alzheimer's for the first time.

Late-onset Alzheimer's, which occurs at age 60 or after, is the most common form of the disease.

A2M is known to interact with a nerve cell receptor called low-density lipoprotein receptor-related protein (LRP). Two clues led the researchers to examine whether A2M mutations might be associated with Alzheimer's risk.

One is that amyloid precursor protein and APOE also interact with LRP. The second is that cell culture studies have shown that A2M tightly binds the protein fragment A-beta, the major component of the amyloid plaques that characterize the brains of Alzheimer's patients.

The cell culture studies showed that A2M helps with the breakdown and removal of A-beta from brain cells. If something goes wrong with that breakdown and removal process, it is believed to lead to Alzheimer's development.

Study co-author Rudolph Tanzi, director of the Massachusetts General Hospital's Genetics and Aging Unit, said the discovery of the A2M mutation adds a new piece to the puzzle of Alzheimer's and suggests how the disease may develop. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 3 August 1998)

Immunity switch activates drug discovery

British researchers say they have identified an "on-off" switch for the immune system, a finding that may open the door to new treatments for diseases like cancer, diabetes, arthritis and multiple sclerosis.

Investigators from Chiroscience Group plc, Cambridge, said they found the gene in mice that have a fatal disorder, in which their immune systems run amok and attack healthy tissues as if they were a virus or other disease causing invader.

Chiroscience researchers have found a similar gene in humans, but so far they do not know how it works and have not found a patient whose immune system is raging as it is in the mice. The disorder only affects males, since the gene switch is found on the X chromosome.

The gene switch appears to regulate certain cells of the immune system, called CD4 T-cells. The Chiroscience investigators have found a mutation that lets the system run out of control, allowing these cells to attack and destroy normal tissues.

A Chiroscience spokesman said that this new gene could prove to be useful in the treatment of diseases where the immune system is too active or too sluggish. In cancer, for example, therapies could be developed to inhibit the gene, strengthening the immune system's attack on tumour cells. In diseases caused by an overactive immune system—the autoimmune conditions like diabetes, Crohn's, rheumatoid arthritis and psoriasis—drugs could be developed to activate the gene or copy its effects in the body. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 5 October 1998)

Tracking cancer's lethal helpers

More than half of all tumour cells contain tiny extra chromosomes that encourage the cancer to grow. Now scientists may be able to find a way to rid cells of the excess DNA, using a technique that lets them watch these chromosomes at work in living cells.

Called double minute chromosomes, the renegade DNA contains genes that have been duplicated and subsequently broken away from the chromosome where they are usually found. Double minute chromosomes have never been seen in healthy cells. "They are absolutely specific to cancer", says Geoffrey Wahl, a cancer researcher at the Salk Institute in La Jolla, California. Without double minute chromosomes, he believes, many tumours would stop growing.

These renegades have been difficult to study, Wahl says, because they are too small to see. Even the largest double minutes are roughly a thousandth the size of a normal chromosome and can be seen only be staining cells. Unfortunately, standard DNA stains are toxic to cells because they damage DNA.

Wahl and his colleagues took advantage of a set of proteins called histones found in all eukaryotic cells. Histones wrap themselves in DNA, condensing the immensely long strands found in a cell's nucleus. The team fused a human histone gene to the gene for the protein that gives certain jellyfish their greenish glow. This made all the chromosomes of cell cultures, as well as the double minutes, glow bright green.

Using the green histones, Wahl's team confirmed earlier reports that double minutes hitch a ride to daughter cells during cell division. Ordinary chromosomes are dragged into each new cell by molecular handles called centromeres, which double minutes lack.

During cell division, the researchers saw double minutes gather together and latch onto the normal chromosomes.

The technique could be adapted to screening for drugs that eliminate double minutes. This would involve applying a battery of drugs to cancer cells into which the green histone gene had been inserted and then monitoring the growth medium. If cells expel their double minutes, the growth medium will fluoresce. (Source: *New Scientist*. 21 March 1998)

High VEGF levels strong sign of more deadly breast cancers

Women with breast cancer who have high levels of a protein that helps tumours create a network of blood vessels are more likely to suffer relapses and die from the disease, new research suggests.

Measuring the protein, known as VEGF, may improve the odds for breast cancer patients because doctors will be able to pick therapies based on the molecular makeup of the individual's tumour, say the investigators.

In two studies, scientists found that women with higher levels of VEGF had a 30 per cent greater chance of having their cancers return after seemingly successful treatment and had three times the risk of dying of the disease, compared to women with less of the protein.

Oncologist Urs Eppenbegger suggests that measuring VEGF and other proteins involved with tumour growth and

spread may be a better way to assess the disease than the factors doctors routinely look at today. These include tumour size, sensitivity to oestrogen and whether the cancer has spread from the breast to the lymph nodes, the scientists say.

VEGF is one of several proteins that promote a critical process known as angiogenesis, in which tumours develop their own system of blood vessels to get nourishment.

Eppenbegger, from Switzerland's Kantonsspital Basel, led a research team that looked at tumours from more than 300 women who had early stage breast cancer. They measured VEGF levels and other proteins that promote angiogenesis and metastasis, or tumour spread.

The scientists said that women who had the highest levels of VEGF and an enzyme called uPA were at a 30 per cent higher risk of having the cancer return about four years after surgery to remove their tumours, when compared to women with lower levels of the protein.

In the second study of more than 500 patients, scientists from the Umea University Hospital in Sweden found that breast cancer patients with higher VEGF levels were three times more likely to die than those with the lowest levels of the protein. (Extracted form *McGraw Hill's Biotechnology Newswatch*, 7 September 1998)

Spoilt division

A microscopic tug of war may be behind genetic confusion in tumour cells.

When healthy cells divide, one copy of each chromosome is pulled into each daughter cell along protein guide wires, or microtubules. In tumours, this often goes awry and cells receive more or fewer chromosomes than their parent. So tumours rapidly reshuffle their genes, a process that makes some cells more aggressive.

Biologists suspected the centrosome, which helps form and organize microtubules, was involved. To test this, Jeffrey Salisbury and his colleagues at the Mayo Clinic in Rochester, Minnesota, took tumour cells from breast cancer patients and stained them with fluorescent antibodies that bind to a centrosome protein called centrin. They found the cells had more than 40 times as much centrin as healthy controls.

That centrin surplus suggested the cells had extra centrosomes, which should recruit more protein guide wires. When the team tested microtubule formation, they found healthy cells generated about five microtubules, tumour cells about 47.

Salisbury believes chromosomes are caught in a tug of war when tumour cells divide, causing the rapid genetic variation. Drugs that slow centrosome replication might make cancer easier to treat, he says. And by counting centrosomes, it may be possible to predict which tumours are stable and which are ready for a genetic shuffle. (Source: *New Scientist*, 28 March 1998)

Kamikaze cells

A protein that is crucial for cell suicide might be a good target for cancer drugs.

Immune cells known as T lymphocytes, which destroy infected or cancerous cells, normally self-destruct afterwards. But Peter Juo and his colleagues at Harvard University produced mutant T-cells that failed to do so. These cells lacked the gene for caspase-8, a protein known to be involved in cell death.

The team concludes that caspase-8 is necessary for T cells to respond to a signalling protein called Fas ligand, which initiates cell death. Some tumours fend off the immune system by making Fas ligand, causing T-cells to die when they try to attack. Juo says drugs that target caspase-8 might be able to stop this. (Source: *New Scientist*, 5 September 1998)

The first gene marker for IQ?

After five years of winnowing through genetic data on groups of normal and gifted children, scientists have identified the first marker for a gene that may influence what psychologists call "g", or general intelligence—the essence of what intelligence quotient (IQ) tests measure. It only accounts for a tiny portion of cognitive ability, but the researchers say it is a step towards the goal of tracing the biochemical pathways between genes and learning.

The researchers, led by psychologist Robert Plomin of the London Institute of Psychiatry, launched their hunt on chromosome 6. They used DNA from 51 children whose IQS averaged 103 and 51 children with a mean IQ of 136. Of the 37 markers looked at, one stood out: a stretch of DNA in *IGF2R*, an insulin-like growth factor receptor gene. Almost all the subjects had one or both of the most common versions of the gene, allele 4 and allele 5. But almost half of the high-IQ group had at least one copy of allele 5, a rate twice as high as in the average group. The finding was replicated in 102 other children, half of them with a superhigh IQ average of 160.

The researchers concluded that a gene very close to this marker, which itself is believed to be non-functional, could account for about 2 per cent of the variance in IQ, or about four IQ points. (Extracted from *Science*, Vol. 280, 1 May 1998)

Sticky problem

The discovery of a gene that helps malaria wreak havoc could lead to more effective treatments for the disease.

The malaria parasite *Plasmodium falciparum* makes a protein called PfEMP1 which turns red blood cells unusually sticky. They bind to blood vessel walls, a process called cytoadherence, and block small vessels. But because the parasite has over 50 different genes that code for versions of the PfEMP1 protein, it seemed impossible to block its production.

Now Donald Gardiner and his colleagues at the Menzies School of Health Research in Darwin, Australia, have found a single gene which seems to work with PfEMP1. When this gene was switched off, cytoadherence ceased.

The gene has been named *clag* (cytoadherence-linked asexual gene). (Source: *New Scientist*, 27 June 1998)

Single amino acid change improves aspirin heart protection

One single change in the P1A gene responsible for blood clotting mechanisms in the body can make the difference between people who are at risk of heart attacks and those who have a far lower risk, according to researchers at Ohio State University.

Dr. Glen Cooke, a post-doctoral student, said the P1A2 polymorphism in which a thymidine protein is exchanged for a cytosine molecule is all that is necessary to make some people have stickier blood platelets.

Sticky blood platelets make clotting easier and heart attacks and strokes more likely. But said Cooke, the same polymorphism that makes a person more susceptible to an unwanted and frequently fatal cardiovascular event also means that the same person who carries the particular P1A2 genetic switch will be able to utilize aspirin better to make platelets "unstickier".

Cooke estimated that a person with P1A2 will get 10 times the benefit of aspirin than a person with the normal gene in cases of heart attacks.

While having the P1A normal gene confers considerable protection against heart attacks, Cooke said that the gene does not prevent heart attacks.

In those people having a heart attack or stroke and who have P1A normal genes, doctors may be better off treating the patients with warfarin or the recently approved anti-thrombotic drug clopidrogel (Plavix, co-marketed by Sanofi of France and Bristol-Myers Squibb of Princeton, NJ).

The gene, situated on Chromosome 17, exon 2, position 1565, makes the T-to-C switch and results in production of proline rather than leucine, Cooke said.

That substitution results in platelets aggregating more quickly to form clots. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 4 May 1998)

Ribozymes nip and tuck damaged genes in sickle cell disease

Duke University Medical Center researchers said they have devised a new gene therapy that is the first step towards an effective treatment for sickle cell anaemia, a painful, inherited condition that shortens life.

The Duke researchers found that by correcting damaged genetic material, they might be able to rid the sickle cell defect from people born with the debilitating disease. That material, ribonucleic acid (RNA), is a chemical that carries and translates genetic information from DNA to areas of cells where proteins are manufactured.

"We have shown for the first time that it is possible to correct a genetic defect in blood extracted from patients", said Dr. Bruce Sullenger, senior author of a paper that appeared in *Science*.

Sickle cell anaemia treatments to date deal largely with symptoms of the disease rather than its underlying genetic cause, said Sullenger, who also is assistant professor of experimental biology at Duke University Medical Center in Durham, NC.

His approach is to actually repair the defect, which in the case of sickle cell anaemia is a single gene.

Sickle cell anaemia is an inherited disease most common among people with ancestors from Africa or the Middle East. About one in 12 African Americans carries the sickle cell trait, and about one in 100 actually gets the disease.

Red blood cells of people with sickle cell anaemia contain an abnormal type of haemoglobin, the molecule that carries oxygen through the body. The defect is caused by a single tiny change in the protein portion of the haemoglobin molecule that distorts the red blood cells into a sickle shape. The sickle cell is fragile and easy to destroy, leading to anaemia.

Complications of sickle cell disease include stroke, bone pain, kidney damage and breathing problems. The disease can shorten life by decades.

Sullenger and his colleagues are using a molecule called a ribozyme, a type of RNA enzyme that can find a specific sequence of RNA code, chemically cut out a section and splice in another.

They devised a way to use this natural process to fix defective RNA. In the case of sickle cell disease, the repair is to a defective globin gene.

For their experiments, the scientists used blood from sickle cell patients and umbilical cord blood, the afterbirth of normal infants. From this blood they isolated precursor blood cells, which produce mature blood cells. They added ribozyme molecules carrying the corrected foetal globin genetic sequence into the cells using liposomes.

Once inside the cell, the ribozymes located the faulty RNA by matching up letters of the genetic code to the defective globin RNA. They then snipped off the defective piece and added in the corrected sequence.

To date, the research has only been on blood in a Petri dish. Within the next year or so Sullenger hopes to start tests on mice, and then after that begin Phase I trials on humans. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 June 1998)

Anthrax toxin turns off MAPK

One of three proteins that form the deadly anthrax toxin has been shown to target the mitogen-activated protein kinase (MAPK) signal transduction cascade. After screening a database of antineoplastic drugs tested against a panel of human cancer cell lines, National Cancer Institute (NCI; Bethesda, MD) investigator Ken Paull noticed that anthrax LF protein had the same inhibition profile as an MAPK inhibitor synthesized by Parke Davis (Ann Arbor, MI). This prompted George Vande Woude's laboratory at the Advanced BioScience Laboratories-NCI, to investigate further. They demonstrated that injection of LF into *Xenopus oocytes* blocks progesterone-induced maturation, a process dependent on the MAPK cascade. By enzymatically cleaving an upstream activator of MAPK, MAP kinase kinase (MKK1), they showed that LF was turning off the MAPK cascade. In addition to revealing useful therapeutics to combat anthrax, Vande Woude believes studies of LF "may allow design of reagents that slow down the MAP kinase cascade, which will give us interesting tools for studying this pathway and, potentially, clinical therapies for cancer". (Source: *Nature Biotechnology*, Vol. 16, June 1998)

Masking against β-thalassemia

A team of researchers at the University of North Carolina (Chapel Hill, NC) and Bern University (Switzerland) has developed a novel antisense approach using small nuclear (sn) RNAs that may provide a useful alternative to conventional gene therapy. The method could eventually be used to treat patients with β-thalassemia, a blood disorder resulting from aberrant expression of the β-globin gene that can be fatal. By hooking up U7 sn RNAs (molecules normally involved in 3 end processing of histone pre-mRNAs) to β-thalassemia antisense sequences, the authors were able to mask aberrant splicing sites in pre-mRNAs, obtaining up to 65 per cent of normal β-globin expression in cell lines. This method—the first in which antisense therapy has been used to restore, rather than downregulate gene expression—could be used to cure not only other diseases caused by splicing anomalies, but also conditions caused by an unbalanced ratio of alternatively spliced mRNAs. (Source: *Nature Biotechnology*, Vol. 16, June 1998)

Double agent

Jumping DNA that may have helped the mammalian immune system evolve also appears to have brought with it a curse, according to researchers in the US. They have found evidence that genes which allow antibodies to mutate and attack new pathogens could also cause mutations that lead to leukemia.

Harmful microorganisms and viruses often mutate far faster than the animals they infect. The vertebrate immune

system can keep up with them only because antibody genes continually change as well. Antibody genes in blood cells called β-lymphocytes mutate before becoming active when special editing proteins remove sections of the DNA. The break points are seldom the same in any two cells, so β cells make a huge variety of antibodies.

David Schatz, a molecular biologist at Yale University, discovered the proteins responsible for rearranging antibody genes around a decade ago. Now his group, along with a team headed by Martin Gellet at the National Institutes of Health (NIH) near Washington, DC, have independently suggested that the genes for these proteins, called RAG 1 and RAG 2, evolved from a transposon, a mobile unit of DNA that can hop into and out of chromosomes. The researchers say that the transposon probably crossed from some other organism to the genome of an ancestral mammal hundreds of millions of years ago.

The teams made the discovery after adding purified RAG 1 and 2 proteins to rings of DNA in a tube. Because the DNA included two copies of the sequence of bases that the RAG proteins bind to, a chunk of DNA containing those binding sites and the sequences between them was cut out.

But rather than remaining separate, the portion of DNA that had been removed was reinserted at a new position in the ring. Schatz says this suggests that the RAG genes originally came from a transposon. "Evolutionary events like this are very hard to prove", he says, but he believes that this is good circumstantial evidence.

The NIH researchers propose that the ability of the RAG proteins to chop out a chunk of DNA and insert it elsewhere in a chromosome could account for certain types of blood cancer. For years, researchers have found that in some leukemia patients, part of an antibody gene has become linked to a cancer gene on a different chromosome. It is possible that the antibody gene activates the cancer-promoting gene.

According to Meni Melek, one of the members of the NIH team, no one has ever been able to explain this unnatural pairing of two gene segments.

Schatz thinks that cells can normally prevent excised DNA being randomly reinserted. But it could be that this mechanism can go wrong, leading to cancer. Schatz adds that the theory is still very speculative at this stage. (Source: *New Scientist*, 5 September 1998)

Improved prospects for transplants

People who desperately need a bone marrow transplant could benefit from a technique that makes a perfectly matched donor unnecessary. If the marrow transplants are successful, then eventually patients who have kidney or heart transplants might be spared a lifelong course of drugs to prevent rejection of their new organ.

Bone marrow stem cells—a type of blank blood cell—give rise to all the different cells that make up the immune system. This means that patients with leukemia and other diseases of these cells can sometimes be cured by destroying most of their existing marrow with radiation and chemotherapy, and replacing it with marrow from a donor.

Unfortunately, donated cells can attack the patient's body, causing life-threatening graft-versus-host disease. This happens when there are too many differences between HLA antigens on the outside of the patient's cells and the donor's cells. HLA antigens trigger an immune response to foreign tissue. Most patients receive bone marrow transplants from a brother or sister, who are more likely to share the six key HLA antigens. But even then, 40 per cent of patients will suffer from graft-versus-host disease. When fewer than four of the antigens match, the death rate is around 80 per cent.

Suzanne Ilstad, a transplant surgeon at the Allegheny University of the Health Sciences in Philadelphia, has come up with an alternative approach. Graft-versus-host disease is triggered by mature T-cells that are transplanted along with the stem cells. So Ilstad has developed ways to screen out these cells, while increasing the proportion of other cells, called facilitating cells, that help the marrow graft into the patient's bones. The technique depends on the ability to recognize molecular markers on the surface of the facilitating and T-cells.

Last April, Ilstad started a clinical trial with desperately ill leukemia patients who had no hope of finding a perfectly matched donor and were willing to risk a mismatched transplant. Of 25 patients taking part in the trial, none has rejected their graft. While the first patients did develop chronic graft-versus-host disease, further refinements of the separation technique, which enable Ilstad to strip out the immune cells, seem to have solved the problem. Ilstad is now planning a larger trial of the technique.

Following her success with marrow grafts, Ilstad has moved on to solid organ transplants. Animal studies have shown that if an organ is transplanted along with marrow from the same donor treated with Ilstad's separation method, the recipient's modified immune system will not attack the new organ.

When other transplant surgeons have tried to give mismatched bone marrow the stem cells failed to graft because researchers had stripped out the facilitating cells along with the damaging T-cells. After reviewing Ilstad's results with the leukemia patients, the US Food and Drug Administration earlier this month decided to let her try the new technique in around a dozen patients receiving kidney or heart transplants. (Source: *New Scientist*, 28 February 1998)

Research on animal genes

Sickle cell mice may lead to new treatments

Genetically engineered mice that mimic all the symptoms of human sickle cell disease were developed by a team led by Edward Rubin and Chris Paszty at LBNL. This new mouse strain, which carries human haemoglobin with no counter-acting mouse genes, provides a means for effective testing of experimental treatments. Each year, sickle cell disease afflicts about 100,000 babies, primarily of African descent, who endure the painful debilitating condition caused by a mutant haemoglobin gene. (Source: *Human Genome News*, 9 (3), July 1998)

Hungry hormones

Brain proteins that make rats guzzle food have emerged. If there is a way to block these proteins it could lead to the development of new appetite suppressants.

Masashi Yanagisawa at the University of Texas in Dallas and his colleagues stumbled across the hormones while synthesizing binding proteins for brain receptors with unknown functions. They called them orexins after the Greek word meaning appetite.

When orexins were introduced into the brains of rats, they consumed more food than normal. Also, if the researchers withheld food from the rats, orexin levels in their brains rose. "The highly specific expression pattern really excites people because it implies that orexins may not have a

lot of other functions", says Yanagisawa. So drugs that interfere with these proteins could have few side effects. (Source: *New Scientist*, 7 March 1998)

Where am I?

Mice that have been genetically altered to make a human protein linked to Alzheimer's disease get lost easily.

Neurologist Lennart Mucke and colleagues at the University of California, San Francisco, replaced the mouse gene for ApoE, a protein that transports lipids in the brain, with one of two variants of the human version, E3 or E4. E4 is associated with a higher risk of Alzheimer's disease, but scientists do not know why.

As they aged, the mice with E4 began to have problems recalling the location of a platform hidden in milky water. But the mice did not develop the tangles and plaques in their brains that are the hallmark of Alzheimer's. (Source: *New Scientist*, 12 September 1998)

Master gene

A genetic master switch for a major component of the immune system has been discovered in mutant mice. The mice were first bred 50 years ago during studies of the effects of radiation as part of the US Manhattan Project to develop nuclear bombs.

Male mice carrying the so-called "scurfy" mutation on their X chromosome die within weeks because T-cells in their immune system mistakenly assault tissue throughout the body. Chiroscience, a company based in Cambridge, told shareholders that it had identified the mutant gene that causes the condition.

Researchers found that the healthy gene reins in activity of the immune system's CD4 cells. These stimulate antibody production and activate killer CD8 cells to combat infections. The gene dampens the activity of CD4 cells once infection is under control. (Source: *New Scientist*, 10 October 1998)

Dung DNA

Biologists have extracted and sequenced DNA from 20,000-year-old dung found in a cave near Las Vegas in Nevada. Hendrik Poinar and his colleagues at the University of Munich in Germany used the DNA sequences to identify the source of the dung as the Shasta ground sloth, now extinct for 11,000 years.

Although digestion had split the DNA into chains of no more than 300 base pairs, Poinar was able to distinguish sloth sequences from the DNA in its food. The researchers report that the sloth's diet included seven families of plants, among them types of capers and mustards, none of which could have been recognized without the DNA analysis. (Source: *New Scientist*, 25 July 1998)

Dinner party gene

A single gene seems to determine whether or not a roundworm prefers to dine alone.

When placed on a dish full of bacteria, roundworm either graze as a herd or strike out in different directions. To find out what controls this innate social behaviour, Cornelia Bargmann and her colleagues at the University of California in San Francisco collected 17 strains of the roundworm *Caenorhabditis elegans* from around the world. They analysed a gene closely related to a human gene that influences appetite.

All 12 social strains had one version of the gene and all 5 solitary strains had another. The difference between them was a single amino acid change in the gene's product. When the researchers changed this single amino acid in the solitary strain, the worms instead ate in groups, proving that a single gene can affect social dynamics. (Source: *New Scientist*, 12 September 1998)

Only the best

Developing sperm cells are screened for genetic fitness before they are allowed to mature—but the selection process breaks down with age. These findings, from a study of mice, could explain why the children of older fathers are more likely to have birth defects.

Biologists have known for some time that in mice, sperm cells tend to have fewer mutations than other cells. Christi Walter of the University of Texas Health Science Center in San Antonio and her colleagues studied mice that had been given an extra gene which, when mutated, produces a protein that turns a gel blue. If the gene is not mutated it has no effect on the mouse. The mutations in the extra gene give a guide to the number arising in the mouse's own genes.

The researchers removed the animals' testes and separated out sperm cells at different stages of development so that they could see how many cells with mutations were present at each stage.

Cells that were at the earliest stage of development were the most likely to carry mutations, the researchers found. Cells that were committed to meiosis—cell division that halves the number of chromosomes in mature sperm—were less than half as likely to carry mutations.

In old mice, the frequency of mutations is up to 10 times that in young ones—suggesting the protection mechanism breaks down with age. (Source: *New Scientist*, 29 August 1998)

Cancer killing frog egg protein has mammalian equivalent

A protein discovered in 1991 in frog eggs proved to be a potent killer of cancer cells, and now a new study shows that a related protein found in mammals has the same power.

Scientists from the University of Madison in Wisconsin report that ribonuclease A, which is a protein produced by the pancreas for digestion, can be genetically altered to kill cancer cells and leave normal cells untouched.

The group's discovery drew upon the 1991 discovery of New Jersey biotech company, Alfacell, which found that a ribonuclease protein in the Northern leopard frog had anti-cancer properties.

That company is now testing a drug based on its discovery, which it calls Onconase. The drug shows promise in mesothelioma, and also inhibits HIV replication, according to an NIH study.

The Wisconsin team used the frog-derived protein as its jumping off point to enquire what made it so effective as compared to a similar mammalian-made protein.

Biochemist Ron Raines compared the molecular structure of the frog protein with a similar ribonuclease protein in cows; the bovine protein is similar to the human one.

Raines discovered that the two differ in their ability to bind with a ribonuclease inhibitor, which is found in almost every body cell, and prevents ribonuclease from attacking and breaking down cellular RNA.

While it is not clear why the ribonuclease attacks only cancer cells, Raines thinks that there are probably unique receptors on the outside of cancer cells that bind more tightly to ribonucleases.

With the bovine form of ribonuclease, the researchers then created two variant strains of the protein that did not bind tightly to the inhibitor. These variants were shown to be lethal to cancer cells in laboratory tests.

Raines' lab is now working to create variant strains of human ribonuclease that can produce the same cancer-fighting effects, which should also be less immunogenic if human-based. (Source: *McGraw Hill's Biotechnology Newswatch*, 7 September 1998)

Hawaiian researchers produce three cloned mice generations

A team of Hawaiian scientists has cloned 50 mice from adult somatic cells using a technique distinct from that used to clone Dolly, the sheep. The mice were created in a series of four experiments from a cumulus cell, which surrounds developing oocytes.

The researchers, led by Ryuzo Yanagimachi, Ph.D., of the University of Hawaii in Honolulu, described the work as "the first successful reproducible cloning of a mammal from adult cells", extending three generations. The cloning method, which involved microinjection instead of electrofusion of nucleus to donor egg, and which has been dubbed the "Honolulu technique", might be more viable for the production of drugs using transgenic animals than earlier techniques because of its efficiency of reproducibility.

The new method has been licensed to Honolulu-based ProBio America, which agreed to allow PPL Therapeutics (Edinburgh, Scotland) to use the new technology for research into cloning pigs and other animals. PPL will be part of an international consortium that will be licensed by ProBio America to use the Honolulu technique. (Extracted from *Genetic Engineering News*, August 1998)

Research on plant genes

Groups map sorghum genes

The National Institute of Agrobiological Resources of the Ministry of Agriculture, Forestry and Fisheries has perfected a gene map of sorghum.

The gene map, expressing the types of genes and their relative positions on chromosomes, revealed that 70 per cent of a sorghum genome has the same structure as the rice genome, and thus, it showed possibilities for applying achievements in the rice genome project to sorghum.

As sorghum is one of the most important feed crops in the world, application of the gene map in the field of breeding for the purpose of improving pest and disease resistance, crop yield and quality is likely to draw attention.

The genome size of sorghum is about twice that of rice.

"It was an achievement brought about by comparative genome studies with a rice genome", said Naoki Katsura, Chief of the National Institute of Agrobiological Resources. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 October 1998)

Plants made resistant to major fungal diseases

Potatoes and tobacco plants have been engineered to carry a gene which make them resistant to four major fungal diseases. Equipped with this gene, the plants make a powerful antifungal enzyme, endochitinase, which digests fungal cell walls and other structures made from the tough biopolymer chitin.

The gene itself comes from the fungus *Trichoderma harzianum*, a parasite which attacks other fungi. Matteo Lorito of the Federico II University of Naples and his team transferred the gene into tobacco and potato plants, and then exposed them to four serious fungal diseases. Between 5 and 10 per cent of the altered plants proved resistant to all four diseases, a trait that was inherited by subsequent generations.

Lorito says that this is the first time a gene of fungal origin has been used successfully to fortify plants against fungal disease. In the past, he says, botanists have loaded plants with plant genes which make antifungal chitinase enzymes, but these provide only weak protection and usually only work against one fungal species.

The endochitinase gene from *T. harzianum* overcomes these limitations because it makes a powerful chitin-digesting enzyme that is lethal to several strains of fungi. The vanquished species include *Alternaria alternata*, which causes severe leaf spot diseases in vegetables and cereals, and its close relative, *A. solani* which causes "early blight" on the foilage of tomatoes, peppers and potatoes.

The gene also protects against *Botrytis cinerea*, which causes "grey mould" on grapes, greenhouse crops and cut flowers, and against *Rhizoctonia solani*, a soil-dwelling fungus that damages root vegetables and grasses. (Source: *New Scientist*, 11 July 1998)

Genetic engineering of potatoes at ARS

Genetic engineering research by scientists with the USDA's Agricultural Research Service should make it easier for potato breeders to sidestep a problem that often frustrates their use of primitive potatoes. The wild plants, native to the Andes Mountains, are a crucial source of genes useful for developing new commercial varieties. However, wild potatoes sometimes contain high levels of unwanted, bitter compounds called glycoalkaloids, which make new varieties unusable.

ARS scientists have discovered a gene that, when reworked, undermines production of an enzyme—solanidine UDP-glucose glucosyltransferase—without which potatoes cannot make a key glycoalkaloid. In laboratory and greenhouse investigations, experimental plants containing the reworked gene—inserted through biotechnology methods—had lower levels of glycoalkaloids. Researchers are seeking a patent for the new gene.

Contact: More information is available on the Web site at http://www.ars.usa.gov/is/AR/archive/dec97/glyc1297.htm (Source: *The AgBiotech Bulletin*, February 1998)

Plant genes for phosphate uptake discovered

Purdue University researchers have isolated genes that help plant roots take up phosphate, a common form of phosphorus. Their research follows observations that phosphorus starvation flips a "genetic switch" that changes certain molecules in the roots of some plants, improving their ability to acquire phosphate.

Working with the Instituto de Recursos Naturales y Agrobiologia in Spain, the researchers at Purdue starved Arabidopsis plants (a member of the mustard family often used as a model system for research) for a week, figuring that this would cause the plants to increase their phosphate uptake mechanisms, which it did.

They then probed the DNA libraries of the starved plants for genes that produce phosphate transporter proteins. They found the genes there, and isolated and decoded them. They also noted that the phosphate-starved plants sent out significantly more messages calling for production of phosphate transporter proteins.

The researchers are now in a better position to understand how phosphorus is taken up by plants, to make changes to the genes involved and to create plants that are efficient acquirers of phosphorus.

Contact: K.G. Ragothama, Tel.: (765) 494-1342; e-mail: ragu@hort.purdue.edu (Source: *The AgBiotetch Bulletin*, February 1998)

Plants infected with viruses used as biofactories

Bioengineered plants infected with special "overcoat-wearing" viruses could be used as biofactories for important proteins such as antibodies, hormones and enzymes, according to an article in the *New Scientist* magazine. The article says that scientists at the Scottish Crop Research Institute (SCRI) have attached proteins to the protein coats of viruses; by infecting plants with these viruses, the researchers are able to produce large amounts of the vital proteins, which remain biologically active while still attached to the virus.

The researchers are modifying RNA plant viruses such as tobacco mosaic virus (TMV) and potato virus X (PVX). Each virus is normally surrounded by 1,500 to 2,500 copies of its coat protein. The scientists have fused a genetic sequence, which encodes the "overcoat", to the virus's protein coat gene. Within 10 days of the virus infecting a plant, millions of its cells each produce up to 1 million modified virus particles. The overcoat can make up as much as 20 per cent of total plant protein. The tests also suggest that virus-infected plants could be used to clean up contaminated land. (Source: *The AgBiotech Bulletin*, February 1998)

New genes for an old crop: genetic engineering in cassava

Cassava is a major tropical food crop, feeding some 500 million people a day. It is a robust crop, tolerant of poor soils and resistant to drought; but it is subject to pests and diseases that reduce yields, and the tubers deteriorate fast after harvesting, limiting their marketability.

Orstom scientists and their colleagues at the International Laboratory for Tropical Agricultural Biology (ILTAB) at Scripps Research Institute, USA, have been developing genetic engineering techniques for cassava. The overall aims are food security for subsistence farmers and possible new prospects for marketing the crop. If the main limitations can be overcome, cassava could become an increasingly important crop in the coming decades. Output could be substantially increased and, with better keeping quality, small to medium-scale commercialization would be possible. By introducing novel traits, cassava could even be made to produce biological substances with added commercial value.

Methods used at ILTAB include microparticle bombardment and *Agrobacterium*, to insert foreign genetic sequences that confer disease resistance.

Cell culture and genetic transformation techniques are now beginning to be used on a routine basis. Scientists have already produced more than thirty plant lines containing a gene for resistance to cassava mosaic virus type CsCMV, which is a big problem in Brazil. Plants containing genes thought to code for resistance to ACMV, the most devastating mosaic virus, have also been regenerated and are now undergoing analysis. And a wild rice gene that confers resistance to rice bacterial leaf blight is being transferred to cassava plants, as a potential new research tool for resistance to cassava bacterial blight.

Hundreds of different cassava cultivars are grown in the tropics, each community having adapted the plant to its own situation and requirements. For a real impact on cassava production at small farm level, therefore, the technology must be transferred to the countries concerned; local scientists can then apply it to local cultivars. The first step in this process is the transfer to national agricultural research programmes and to the IARC centres CIAT and IITA. Scientists from Zimbabwe, Côte d'Ivoire, Cameroon and Thailand are already training at ILTAB. (Source: *Orstom Actualités*, No. 55, 1998)

The killer within

Washing salad vegetables does not guarantee protection against food borne pathogens, say Japanese researchers. They have found that *Escherichia coli* 0157 can find its way inside plant tissue.

Radish sprouts used as salad toppings are thought to have caused the 1996 outbreak of *E. coli* 0157 in Japan that killed 11 people and gave thousands more diarrhoea. Masaaki Iwaki and his colleagues at the National Institute of Infectious Diseases in Tokyo have been investigating what happens to radish plants grown from contaminated seeds.

The researchers soaked the seeds for eight hours in a solution spiked with the bacteria, and then placed the seeds in sterile water and allowed them to germinate and grow for seven days.

When Iwaki and his colleagues cut cross sections of the plants, exposed them to a fluorescent antibody for *E. coli* 0157 and examined them under the microscope, they saw telltale fluorescence.

The researchers then took contaminated radish sprouts and dunked them in a mercury chloride solution for 10 minutes. When whole sprouts were put on a culture medium, no bacterial colonies grew, but if the disinfected sprouts were cut open, bacteria started to grow. To prevent infection, says Iwaki, it seems that people must either cook radish sprouts or ensure that seeds are uncontaminated.

The findings are "very disturbing", says Thomas Breuer of the Centers for Disease Control and Prevention in Atlanta, who has traced outbreaks of *E. coli* 0157 to alfalfa sprouts. Irradiating seeds could provide some protection, he says, but this interferes with germination. (Source: *New Scientist*, 21 March 1998)

Carrot power

Crop plants that are vulnerable to frost damage can now be protected with an antifreeze protein isolated from carrots.

A gene in carrots makes a protein that stops ice crystals growing in the vegetables. Maggie Smallwood and her colleagues at the University of York isolated this gene and transferred it to tobacco plants.

The researchers say that carrot antifreeze was more stable and effective in tobacco than antifreeze proteins made by fish genes, which had previously been introduced into plants.

"The protein has evolved to work in a plant, so it's more likely to work than a protein that works in the blood of fish", says Smallwood. It also sidesteps ethical objections to placing fish genes in plants. (Source: *New Scientist*, 10 October 1998)

Going to seed

Freakish tissue growing out of leaves has helped botanists pinpoint a "master gene" that controls embryo development in plants.

A team led by John Harada of the University of San Francisco at Davis engineered *Arabidopsis thaliana*, a species of cress, so that a gene called *LEC1* was switched on in all

tissues. Normally, *LEC1* is only expressed in seed development.

Embryonic tissue grew on the plants' leaves, with emerging roots and shoots visible.

The discovery could lead to the development of plants that produce oils and proteins in their leaves that are normally only found in seeds. Farmers could harvest the products sooner, avoiding the delay while seeds form. (Source: *New Scientist*, 4 July 1998)

Greening the desert

Too much sun can be as damaging to plants as too little. Now researchers in Japan have found a way to help plants survive better in deserts and areas with lots of sun by neutralizing the active oxygen species (such as hydrogen peroxide) that kill plant cells. They hope their technique will make it easier to grow food and fruit in desert areas.

In strong sunlight plants close their stomata to prevent water from evaporating. But this also slows down photosynthesis, since less carbon dioxide gets into leaves, and cells die because the energy that would have been used in photosynthesis is used to create active oxygen.

Scientists at the Research Institute for Innovative Technology of the Earth (RITE) and the Agriculture Department at Kinki University in Osaka have found a way to dissolve this active oxygen.

They injected an enzyme-producing gene from bacteria which live in the human colon into the chloroplast of a tobacco plant. The enzyme, called catalase, turns the active oxygen into water. The researchers tested their altered plant by putting it side by side with an unaltered plant under light from six 450-watt sodium lamps. While the altered plant thrived, the ordinary plant withered after two days.

Although tobacco is not an appropriate plant for greening deserts, it was easy to work on because the plant has been widely studied and genetically manipulated, says chief researcher Ken'ichi Tomizawa. He says local standards of living will dictate the type of plant they use. "Developing countries need food plants, but countries like Israel need other types of plants, such as fruit or cash crops." (Source: *New Scientist*, 18 July 1998)

New gene killer

Toxic proteins from a luminous bacterium that lives inside the gut of a worm could protect the next generation of genetically engineered crops from insect attack, according to US researchers.

They say that the proteins could be used as an alternative to the Bt toxin, which has already been engineered into crops such as cotton and maize. Bt successfully kills pests but widespread use has raised fears that insects will become resistant to it. The new system, based on proteins extracted from *Photorhabdus luminescens*, could be alternated with Bt to prevent insects from developing resistance to either, the University of Wisconsin researchers claim.

Photorhabdus luminescens lives inside the gut of a tiny roundworm. When the worm is swallowed by a caterpillar, *P. luminescens* enters the larva's stomach. There it begins to pump out its toxins, which destroy the gut wall and kills the caterpillar within 48 hours. The worm and bacteria then eat the caterpillar corpse.

Richard Ffrench-Constant and colleagues have isolated four toxins from the bacterium and identified the genes that code for them. Industrial partners at Dow Agrosciences are working on genetically engineering the toxin genes into crops, turning them into deadly meals for hungry caterpillars.

A peculiar side effect of *P. luminescens*' attack on a caterpillar is that it emits light along with its toxins, causing the dying caterpillar to glow. However, this effect will not be transferred into crop plants. (Source: *Chemistry & Industry*, 6 July 1998)

Common bond

Immune systems in plants are switched on and fortified by nitric oxide, a gas long known to do the same job in animals.

In mammals, nitric oxide plays a key role in the transmission of nerve signals, but its main task is to orchestrate the first line of defence against infections. Now, two groups of botanists have independently shown that nitric oxide also plays a key role in the immune systems of plants. This suggests that plants and animals inherited the core components of their immune systems from a common evolutionary ancestor.

Daniel Klessig of Rutgers University in Piscataway, New Jersey and his colleagues made their discovery by comparing normal tobacco plants with strains that are unusually resistant to the tobacco mosaic virus, which causes spots on tobacco leaves. When they exposed the resistant strain to the virus and examined plant tissue, the researchers found five times as much nitric oxide synthase, the enzyme that manufactures nitric oxide, as in the normal strains. This strongly indicates that nitric oxide plays an important role in the immune system.

To follow up the result, Klessig's team dosed the leaves and individual cells of tobacco plants with nitric oxide synthase from rats, together with a substance that drives the enzymes. They found that this activated a gene called *Pathogenesis related-1 (PR-1)*, which regulates plant immunity.

These findings are complemented by those from a separate study by Chris Lamb and his colleagues at the Salk Institute in La Jolla, California, which showed that in soya bean cells, nitric oxide sets off a chain of biochemical commands almost identical to that tripped by nitric oxide in neutrophils, white blood cells that engulf and destroy invading bacteria. The cells do this with reactive oxygen intermediates, powerful chemical agents such as hydrogen peroxide and superoxide ions, which are thought to deliver the killer punch. (Source: *New Scientist*, 15 August 1998)

A genetic accident allowed plants to conquer the land

Land plants may owe their evolutionary success to the accidental duplication of a gene early in their history, say scientists in the US and Germany.

The gene in question codes for actin, a key component of the internal skeleton of cells. Like animals, higher land plants have many different actin genes, and the different versions of the protein have different properties. This diversity helps cells perform specialized functions, an essential step in the evolution of complex multicellular organisms.

For years, however, researchers have debated whether the variety of actin genes was what enabled land plants to diversify, or whether the genes diversified later.

Now researchers believe they have evidence to support the first theory. Debashish Bhattacharya of the University of Iowa in Iowa City and his colleagues sequenced the actin genes of three primitive land plants, and several green algae.

The green algae have a single version of the gene, they found, as does *Mesostigma viride*, a single-celled organism that represents the lowest branch on the evolutionary tree leading to land plants. In contrast, all the other primitive land plants they studied have at least two actin genes—evidence that the gene was duplicated around the time the lineage evolved.

Higher up the tree, more duplications appear, suggesting that gene duplication and divergence were key events in the origin of complexity in land plants. (Source: *New Scientist*, 29 August 1998)

Engineered trees in the R&D pipeline

Scientists at Union Camp, Westvaco, and other paper companies are engineering sweet gum, paulownia, cottonwood, among other trees, hoping to create a supertree—one that grows faster than normal but retains hardiness. So far, they have only achieved rapid growth and delicateness—tall, fast-growing specimens that require special treatments like fertilizing, pruning, and weed control. Back to the drawing board.

Other scientists have had more success engineering trees to control weeds and insects that plague tree plantations. The Oregon State University Tree Genetic Engineering Research Cooperative—a consortium of companies, government agencies, and universities—has engineered hybrid poplars to resist the herbicide glyphosate and produce insecticidal Bt toxins. Glyphosate, which is toxic to ordinary poplars, is not used in growing trees except to clear sites. However, with glyphosate-resistant trees, growers could spray plantations with glyphosate. The scientists have also engineered hybrid poplars to produce Bt toxin—in the hopes of controlling a serious leaf-eating pest, the cottonwood leaf beetle. The Cooperative so far has not applied for commercial permits for the engineered trees.

Californian and Swedish scientists are trying to speed up traditional tree breeding by transferring a gene that accelerates flower development from *Arabidopsis* into the European aspen. (*Arabidopsis* is a small, rapidly growing annual plant from the mustard family which is widely used as a model system in plant genetics research.) Typically, an aspen is 10 to 20 years old before it produces flowers, which are essential for traditional breeding crosses. With the gene from *Arabidopsis*, scientists hope to produce aspens that begin flowering at much younger ages.

Sources: Annual report 1996-97, Tree Genetic Engineering Res. Coop., Forest Research Lab., Oregon State Univ., Corvallis; A. S. Moffat, "Genetic engineering turns to trees", *Science* 271:761, 3/9/96; J. Welsh, "Tinkering with genes to get a tall, strong 'super tree'", *Wall Street Journal*, pp. B1, 18, 1/13/98. (Source: *The Gene Exchange*, Summer 1998)

Another weapon against insect pests

Farmers may soon have another genetic weapon in their war against insect pests. Crop plants engineered to produce the bacterial insecticide *Bacillus thuringiensis* toxin, or Bt, may be joined by plants that make proteins called Pht that are also highly toxic to insects. They are produced by the bacterium *Photorhabdus luminescens*, which lives inside nematode worms in the blood of insects.

Richard Ffrench-Constant and his colleagues at the University of Wisconsin in Madison report they have identified four genes responsible for producing the Pht toxins. Two account for most of the lethal effects on tobacco hornworm, making them good candidates for genetic engineering leading to crop plants that produce the toxins directly.

This would give farmers an alternative to transgenic Bt-producing plants. By alternating Bt and Pht, they may be able to delay the evolution of insects resistant to either toxin. (Source: *New Scientist*, 4 July 1998)

Research on viral genes

Rebel virus

The discovery that adenoviruses may cause serious heart inflammation could further jeopardize their role as vectors in gene therapy research.

Researchers have made genetically altered versions of these viruses, hoping to use them as a means of ferrying genes into patients to treat diseases from cystic fibrosis to Alzheimer's. In 1996, scientists at Oxford University found that an adenovirus vector appeared to cause brain damage in lab animals. The virus had been altered so that it could not replicate, but gene therapists suspected that the adenovirus triggered an aggressive immune response, causing dangerous inflammation.

Now Robert McCarthy of Johns Hopkins Medical Institution in Baltimore, MD, and his colleagues have linked an adenovirus with life-threatening heart inflammation. They compared heart tissue from seven myocarditis patients with tissue from six controls. Four of the patients carried an adenovirus, they said this month at a meeting of the American Heart Association in Florida.

Andrew George, who is developing gene therapy at the Royal Postgraduate Medical School in London, says the results raise concern. He hopes other vectors being investigated, such as liposomes, may be safer. (Source: *New Scientist*, 29 November 1997)

Blocking the paths of twin proteins might put a stop to Ebola

A gene with a split personality could explain why the Ebola virus is so dangerous, according to a team of researchers in Michigan. They say their work could lead to a treatment for Ebola infection, and that the schizoid gene could be tamed for use as a tool to treat other diseases.

More than half of people infected with the Ebola virus die within weeks. In these victims, the virus evades the immune system and causes massive bleeding that throws them into shock. Scientists have not found any effective treatment.

Ebola's ruthlessness is all the more remarkable considering that its genome consists of only seven genes. Scientists trying to make Ebola vaccines are especially interested in the gene that encodes a glycoprotein (GP) that coats the virus. By a clever genetic trick the virus uses the same gene to produce a soluble and free-floating version of the protein, dubbed sGP.

Like most researchers, Gary Nabel of the University of Michigan in Ann Arbor had assumed that the free-floating sGP was simply a decoy to distract immune cells.

First the researchers mixed purified sGP with different types of cells. Strangely, the protein seemed attracted only to neutrophils, a type of immune cell that the virus was not thought to infect. Further investigation revealed that a solution of sGP hindered neutrophil activation, a key part of the immune system's early response to a foreign body. Nable says this might be why the Ebola virus spreads so rapidly through the body. "Neutrophils are front-line soldiers of the immune system", he says.

But with the other GP it was different. The researchers attached this protein to an unrelated leukaemia virus to see which type of cells the protein would help it infect. To their surprise, the virus shunned neutrophils and instead went for endothelial cells, which form the walls of blood vessels.

Nabel points out that if GP leads the Ebola virus to these cells, the resulting infection might weaken blood vessel walls, which would explain how the virus causes bleeding throughout the body. The researchers now want to determine if the two GP proteins act the same way in living animals during the course of an infection. If so, then blocking both GP from binding to their targets might stop an Ebola infection in its tracks.

Nabel thinks that GP might also be put to good use in gene therapy. His group has also been experimenting with ways of getting genes into endothelial cells to treat cardiovascular disease, the top killer in the developed world. Engineered viruses sporting GP may do the job more easily than the mechanical methods now used, he says. "We could take advantage of nature and let the protein home in on the right cells". (Source: *New Scientist*, 21 February 1998)

Dubious organs

The discovery of a previously unknown virus in pigs has rekindled fears about the safety of xenotransplants—transplants of animal organs into human patients. The virus, which caused deformities and stillbirths among pigs, also infected two piggery workers who developed severe flu-like symptoms.

One of the biggest question marks over the future of xenotransplants, especially the use of pig organs, is the potential for introducing animal viruses into the human population. Proponents of cross-species transplants say that the organs can be screened for pathogens before use.

Peter Kirkland, from the Elizabeth Macarthur Agricultural Institute in Camden, New South Wales, is a member of a large scientific team which tracked down the cause of a disease which swept through a piggery near Sydney last year. The culprit was a paramyxovirus—which the team traced to a colony of grey-headed fruit bats that were living near the piggery. They believe the bat is the host for the disease.

No sows or growing pigs showed any outward sign of illness. The virus only attacked pig foetuses, which were either stillborn or had serious defects of the brain and spinal cord never before seen in pigs. Although the virus seems to have been contained, "there is nothing to say it won't break out again at this piggery or elsewhere", warned Kirland.

The public health risk is minimal, he said. The virus has not been found in pig products, and none of the 350-plus vets, pathologists and abattoir workers who may have been exposed to infected pigs has contracted the virus. The two infected piggery workers have recovered. (Source: *New Scientist*, 28 February 1998)

Know your enemy

A case of molecular mistaken identity may explain why a viral infection can provoke a body's immune system to destroy its own tissues, say researchers at Harvard Medical School in Boston.

Harvey Cantor and his colleagues studied a strain of mice in which infection by a herpes virus triggers an autoimmune disease called herpes stromal keratitis, a degeneration of the cornea that is a common cause of blindness in humans. They found that the immune system's T-cells, which attack the cornea, recognize two interchangeable targets: a fragment of protein on the surface of corneal cells, and an almost identical one produced by the herpes virus.

This suggested that the corneal cells are innocent victims caught in the crossfire as T-cells fight the viral infection. To confirm this, Cantor's team modified the virus so that it produced none of the crucial protein fragments. They found that the modified viruses did not cause keratitis.

They also showed that when mice that could not produce T-cells were infected with herpes, they remained free of keratitis. But the disease developed normally if T-cells were transfused into the mice.

The results provide the first clear proof of a connection immunologists suspected for years, says Vipin Kumar of the La Jolla Institute for Allergy and Immunology in California. This may encourage researchers to look for similar causes for autoimmune diseases such as multiple sclerosis, Cantor says. (Source: *New Scientist*, 7 March 1998)

Novel treatment may defend the immune system against the worst ravages of HIV

Gene therapy could prevent HIV from wrecking the immune system, a unique study on identical twins suggests. Preliminary results from the trial indicate that such treatment might ward off the opportunistic infections that eventually kill people with AIDS.

Richard Morgan of the National Institutes of Health near Washington, D.C., has genetically modified a harmless retrovirus to carry genes that disrupt two proteins, called Tat and Rev, which HIV needs to preproduce. When the modified retrovirus enters CD4 cells, which are usually killed by HIV, it produces an altered form of Rev that interferes with the protein's ability to move viral RNA from the cell's nucleus into the cytoplasm. It also produces an "antisense" RNA sequence that stops Tat binding to a sequence of viral RNA.

In August 1996, Morgan and his colleagues Robert Walker, Clifford Lane and Michael Blaese persuaded eight pairs of identical twins to join their trial. One of each pair was HIV-positive. This gave each HIV patient a ready source of genetically identical CD4 cells that were free from HIV.

The researchers took CD4 cells from each healthy twin and infected half the sample with the modified retrovirus carrying the therapeutic genes. The other half was exposed to a similar retrovirus carrying only a marker gene so the researchers could see how long the cells lasted. Then all the cells were transfused into the twin with HIV.

Morgan and his colleagues took regular blood samples from the patients, recording the relative quantities of the two types of retrovirus-infected cells. The cells with the therapeutic genes clearly survived much better in seven out of the eight patients. After several months, there was a 20-fold difference in one case.

If the technique becomes generally accepted, Morgan says that a patient's own cells would be used. But while Morgan's retrovirus will ferry therapeutic genes into CD4 cells in the test tube, it will not do so efficiently if simply injected into the bloodstream.

Ultimately, Morgan would like to eliminate the laborious step of removing CD4 cells from the body and culturing them in the lab. This would mean using a genetically modified virus to seek out and infect CD4 cells in the body. One interesting possibility, Morgan says, would be to "take HIV and turn it against itself". (Source: *New Scientist*, 21 February 1998)

AIDS drugs release trapped immune cells

During HIV infection, white blood cells become trapped in lymph nodes and other inflamed tissue rather than being destroyed, a new report argues. This suggests that the immune systems of people undergoing drug therapy could recover more fully than scientists had believed.

Previously, many researchers thought that HIV infection leads to the wholesale destruction of the CD4 white blood cells that orchestrate the immune system, and that to fight back, the body has to grow new ones.

But when Frank Miedema at the Central Laboratory of the Netherlands, Amsterdam and his colleagues measured the rate at which CD4 cells reappear in patients' blood after aggressive anti-HIV therapy, their return was unexpectedly rapid. Miedema says this could be explained only by the release of existing cells from tissues, not by the production of new ones. He suggests that the redistribution of cells occurs because of an imbalance of immune-regulating chemicals called cytokines after HIV infection.

These results, along with those of a T-cell study by French researchers, "provide the final nails in the coffin" of the idea that T-cells vanish because of the death of HIV-infected cells, says Mario Roederer of Stanford University, California, in an accompanying article.

"These results suggest that people on drug therapy, even those with very low CD4 counts, may see their counts rise steadily over a period of years", says Andrew McMichael, an HIV specialist at Oxford University. But he cautions that the extent to which the immune system may recover is unclear. (Source: *New Scientist*, 28 February 1998)

Halting HIV

Molecular sleuthing has uncovered a new class of chemicals that may arrest HIV's spread in the body.

In the past two years it has emerged that before the virus can infect a cell it must latch onto one of several chemokine receptor molecules on the surface of immune cells, as well as a receptor called CD4. Last year researchers reported progress in developing chemicals that block one of these receptors, called CCR5.

However, such drugs would have no effect on M-tropic HIV strains, which use a receptor called CXCR4 to invade cells. Now researchers at INSERM, the French medical research centre in Paris, have made chemical analogues of parts of the chemokine that naturally binds to CXCR4. They report that one analogue they call L5H blocks *in vitro* infection of immune cells but does not seem to stop the receptors transmitting normal chemical signals.

The discovery might eventually lead to drugs that halt CXCR4-reliant HIV strains without impairing the immune system.

Virologist Robin Weiss at the Institute of Cancer Research in London stresses that such drugs for M-tropic HIV strains would not prevent HIV infection or provide early treatment, because CCR5 or "T-tropic" strains of HIV predominate early in infection. These later mutate into the M-tropic form. The French team concludes that both types of receptor—and therefore both strains of HIV—should be targeted. (Source: *New Scientist*, 21 March 1998)

Newborn babies cannot cure themselves of HIV after all

Babies who somehow rid themselves of HIV infection shortly after birth have intrigued AIDS researchers for about ten years. If the secret behind these infants' ability to shake off the virus could be uncovered, it might lead to new drugs or vaccines. But now a team of researchers in the US has come to a startling conclusion about these so-called transient HIV infections—they never actually happened.

Instead, in every case they examined, they believe that sloppy testing probably resulted in an uninfected newborn being misdiagnosed as HIV-positive. Months later, when the test was repeated, the child appeared to have fought off the virus (*This Week*, 8 April 1995, p. 6).

Diagnosing HIV infection in infants is notoriously hard. The standard adult test for anti-HIV antibodies does not work, because maternal antibodies persist in the blood for over a year after birth. So doctors use other techniques such as looking for viral proteins directly, or by using the polymerase chain reaction (PCR) to amplify one viral gene in a sample so there are millions of copies that can be detected.

Lisa Frenkel of the University of Washington in Seattle and researchers report their analysis of 42 instances of transient infection in infants and one unusual case in a new mother. In each case they discovered something suspicious.

In six cases they analysed human instead of viral genes and found the HIV-positive samples belonged to another child. In 17 instances, the virus in the babies' blood was unrelated to the mother's infection, which suggested the sample was infected in the lab. In 20 cases, they could not detect a full set of viral genes in the blood sample. That suggests the original tests were false positives or had been contaminated during PCR.

The researchers conclude that there are problems with these complicated tests for infants and their mothers. In this study they went to great lengths to avoid contamination. For instance, samples from mothers and infants were analysed by different people in different buildings a mile apart.

Frenkel adds that it is still possible there are real cases of transient infections. (Source: *New Scientist*, 23 May 1998)

Gene discovered that retards transformation of HIV into AIDS

A research group of Assistant Professor Kei Tashiro and others of the Genetic Experiment Facility, Kyoto University, recently clarified that the intercellular transmitter (chemokine) "stroma-cell-derived factor SDF-1" functions as one of the genetic factors which controls the period of time from infection with HIV (human immunodeficiency virus) to the development of AIDS (acquired immunodeficiency syndrome).

The research group analysed clinical data in the US and elucidated the correlation between the cases in which the time to development of AIDS is retarded from infection with HIV as a result of "SDF-1 genetic polymorphism".

Although SDF-1 has been reported to suppress the HIV infection *in vitro*, whether it actually controls development of AIDS in a human being has not been proved. Nonetheless, the understanding of AIDS development has been deepened. At the same time, the prospects of developing a new AIDS suppression method using the SDF-1 protein and its affinitive substances seem to be expanding.

For further information, contact the Life Sciences Division, Research and Development Bureau, STA. Tel.: 03-3581-5271, ext. 442. (Source: *STA Japan*, July 1998)

Piggyback ribozymes

Researchers at the Beckman Research Institute of the City of Hope (Duarte, CA) have found a way to sneak an antiviral agent that interferes with viral replication into

virions. By attaching an antiviral ribozyme to $tRNA_3Lys$—a host cellular factor that is recruited by HIV to facilitate DNA replication—John Rossi and colleagues set out to determine whether the ribozyme could be brought in proximity to viral target sequences. Sure enough, when cotransfected with HIV proviral DNA the chimeric $tRNA_3Lys$-ribozyme was packaged into virions bringing the ribozyme to its RNA target. More importantly, the $tRNA_3Lys$-ribozyme was effective in reducing the titre of infectious virions. Rossi speculates that once a chimeric $tRNA_3Lys$-ribozyme is packaged into a virion "it is able to inhibit HIV replication probably through a combination of ribozyme cleavage of target HIV RNA, and blockage of the primer binding site to replication machinery". The group plans to develop this novel strategy into a gene therapy to be combined with existing ribozyme-based gene therapies to reduce HIV infectivity. Source: *Nature Biotechnology*, Vol. 16, July 1998)

Natural AIDS immunity found in people with normal co-receptors

American researchers are studying two groups of people who appear to have a natural immunity to HIV, although they have normal CCR5 and CXCR4 receptors, the 12th World AIDS Conference was told.

The cases are reminiscent of the HIV-resistant Kenyan prostitutes that have been studied for several years by Canadian researcher Francis Plummer of the University of Manitoba and colleagues at Nairobi University.

Sharon Stranford, a postdoctoral fellow at the University of California at San Francisco said she has found 60 high-risk individuals who remain uninfected despite repeated exposure to HIV, and researchers at the Centers for Disease Control in Atlanta report on a group of Thai prostitutes who are also HIV-resistant.

Both groups think that unusual CD8 T-cell responses may be responsible for the immunity.

Stranford and colleagues began their study by looking for high-risk people—defined as people who shared needles or had unprotected sex several times with someone known to be infected with HIV. But despite their repeated exposures, they did not have antibodies to HIV in their systems and also had no detectable virus.

Stranford said that her group had CD8 cells that suppressed viral replication without killing infected cells—a phenomenon found in long-term survivors, as well. She speculates that if that response occurs early—before wide-spread systemic infection—it might be the process of protection.

The CDC group, working with the Thai Ministry of Public Health, found that their women had CD8 T-cell responses to HIV that were weaker and to fewer regions of the virus than were found in infected women. (Source: *McGraw Hill's Biotechnology Newswatch*, 20 July 1998)

HIV joins the superbug set

A strain of HIV resistant to all the protease inhibitor drugs now on the market has turned up in San Francisco.

Protease inhibitors, which disable an enzyme vital for the virus's replication, have dramatically lowered death rates from the disease. Strains of HIV resistant to individual protease inhibitors have shown up before, but AIDS researchers had hoped that any mutations that made a virus resistant to a range of the drugs would also cripple it so badly that it could not be transmitted.

That hope has been dashed by a team led by Frederick Hecht of the University of California at San Francisco. Hecht and his colleagues are studying recently infected patients about to begin drug therapy. Among 35 patients analysed so far, they have discovered one man infected by a virus strain with a wide range of drug resistances. The HIV in his blood contains seven mutations that enhance resistance to protease inhibitors, and four known to boost resistance to drugs that inhibit another HIV enzyme called reverse transcriptase. In laboratory tests, this virus withstood all protease inhibitors on the market at doses up to 12 times those recommended for treatment.

The researchers have analysed the virus from the man's sexual partner and found a nearly perfect genetic match, confirming him as the source. This man had sporadically taken nine antiviral drugs to treat his HIV infection, including protease inhibitors. Breaks in his therapy might have allowed the virus to evolve multiple resistances. "This study shows that we can do more harm than good if we don't help patients take their medications correctly", says Margaret Chesney, a member of Hecht's team. (Source: *New Scientist*, 4 July 1998)

Strike two

Some strains of HIV launch not one but two strikes at the immune system. This may help explain the sudden steep decline of some AIDS patients who have previously been stable and relatively disease-free.

The second strike hits white blood cells known as CD8 cells. Unlike their sister CD4 cells, which are the ones actually invaded by HIV, CD8 cell counts do not decline steadily during the course of HIV infection. Instead, in nearly half of patients, the CD8 count begins to tail off a year or two before the patients take a turn for the worse.

HIV causes this decline in CD8 cells through mistaken identity, according to Eric Verdin, a viral immunologist with the Gladstone Institute of Virology and Immunology at the University of California, San Francisco and his colleagues.

The surface of CD8 cells bears a receptor molecule called CXCR4, which is also found on CD4 cell surfaces. HIV sometimes uses CXCR4 to enter CD4 cells. Verdin's experiments with white blood cell cultures show that when HIV interacts with this receptor on CD8 cells they undergo apoptosis or programmed cell death.

Viral strains that target CXCR4, if they appear at all, do so relatively late in infection. To Verdin, this suggests that the shift to the new strain may cause the drop in CD8 cells. (Source: *New Scientist*, 12 September 1998)

Self help

Hopes of protecting people against some of the most dangerous sexually transmitted diseases have been boosted by the first test of a new method of immunization. A team in Montana has immunized mice against *Chlamydia*, a bacterium that can cause infertility in women. The same approach might even work against HIV.

Until now, vaccines have been notoriously unsuccessful at stimulating immune responses in the mucous membranes of the body, the route through which HIV, *Chlamydia* and other sexually transmitted pathogens enter the body. And though vaccines are good at stimulating antibody-producing B cells, they are poor at triggering T-cells to destroy infected cells. T-cells are vital in the fight against *Chlamydia* and HIV.

Harlan Caldwell and his colleagues at the Rocky Mountain Laboratory of the National Institute of Allergy and

Infectious Diseases (NIAID) in Montana tried a different approach. They took bone marrow from female mice and cultured dendritic cells by adding interleukin-4. Dendritic cells recognize foreign molecules and recruit other cells of the immune system to attack invaders. The team added heat-killed *Chlamydia* to the culture to sensitize the dendritic cells to the bacterium and then put the cells back into mice.

This technique is already used against some cancers, where dendritic cells are sensitized to cells taken from the patients' own tumour. But this is the first time it has been used to immunize against infectious disease.

When the immunized mice were later exposed to live *Chlamydia*, their response was as vigorous as that of mice immune to the disease because of a prior infection, and none developed signs of disease.

Caldwell is optimistic that the technique will work in people. Some form of vaccination is badly needed, as a recent study of army recruits has suggested that rates of chlamydial infection among young women in the US are as high as 10 per cent. No clinical trials have yet begun, though a test in primates is already under way.

For a high-profile and deadly disease such as AIDS, even the unconventional approach may be worth the effort and expense. Several laboratories are already trying to use isolated dendritic cells to stimulate immunity to HIV, says Anthony Fauci, Director of the NIAID, based at its headquarters near Washington, D.C. Caldwell's results prove that the concept works, he says. (Source: *New Scientist*, 26 September 1998)

Research on the common cold virus

Researchers in the US have found that parts of the protein shell of the common cold virus flap open in a motion they call "breathing"—and compounds that stop viruses infecting cells can stifle this flapping. The discovery could lead to rapid screening methods for antiviral drugs.

A virus only shows real signs of life after it has infected a cell. Once inside, the invader sheds its protective protein coat, releases its genetic material and hijacks the cell's resources to replicate itself. But according to the textbooks, viruses shut down between infections, becoming inanimate.

Until now, many scientists were convinced that the fluctuations in viral shape that happened outside the cell were probably minor and of little importance. So when Thomas Smith, a biochemist at Purdue University in Lafayette, Indiana teamed up with chemist Gary Siuzdak and his colleagues at the Scripps Research Institute in La Jolla, California, to probe the motions of the virus coat, the best he hoped for was to reveal a tiny twitching. The researchers treated the virus with an enzyme that chops up proteins. They reasoned that if the breathing effect was only small, exposing just the outermost atoms of the virus's coat, then the first wounds the enzyme inflicted on the virus should be in these layers.

In most cases, this turned out to be true. But to the team's surprise, one protein, called VP4, was also an early victim—even though previous studies showed that it was usually buried inside the virus, 25 atom lengths from the surface.

The researchers also studied the effect of WIN 52084, an experimental drug discovered in a search for compounds which might stop the cold virus infecting human cells The breathing effect stopped in the presence of the drug, and the virus took up to 18 hours to succumb to the enzyme's attack, rather than the minutes it would usually take.

The researchers suspect the drug is effective because when the surface cannot breathe, the virus cannot release its genetic cargo. This suggests that enzyme digestion tests could replace laborious cell infection experiments as a first screen for promising new drugs to combat the cold virus—and perhaps other viruses as well. (Source: *New Scientist*, 20 June 1998)

DIY molecule

A virus has provided a hint of how the Earth's first organisms got things done.

Many biologists believe that the earliest forms of life comprised molecules of RNA—DNA's chemical cousin—that were able to copy themselves. Now Steven Lommel, a virologist at North Carolina State University in Raleigh, suggests how RNA itself may have regulated that copying. His team reports the discovery that a modern virus still uses this type of regulation.

The researchers discovered that a tiny snippet of RNA in the red clover necrotic mosaic virus directly turns on a gene for making the virus's protein casing. Normally, proteins turn genes on and off. But the virus is still modern compared to the theoretical early life forms—it requires an enzyme to copy its RNA. (Source: *New Scientist*, 15 August 1998)

Research on bacterial genes

Tricksy TB gene

A gene that helps tuberculosis to outwit immune defences has come to light.

The immune system relies heavily on white blood cells called macrophages to kill invading bacteria. Yet some TB strains shrug off the attack.

A team led by Lee Riley of Cornell University in New York suspected that a gene in *Mycobacterium tuberculosis* called *nox*R1 was responsible. To test this, they spliced the gene into *E. coli* and *M. smegmatis*.

These bacteria then had improved resistance to the defences of mouse macrophages. If the same effect occurs in humans, this could lead to new TB treatments. (Source: *New Scientist*, 13 December 1997)

New bacterium compared to Bt

For 30 years Bt, a bacterium with remarkable insecticidal properties, has been an environmentally friendly pest control mainstay. As a form of biological pest control, it is the only bacterium from which widespread commercial applications have been possible. It has also been widely used in plant breeding to add insecticidal properties to plants.

Now a team of scientists from two laboratories at the University of Wisconsin-Madison, working in collaboration with scientists from DowElanco of Indianapolis, have identified a new bacterium with similar insect-thwarting properties. *Photorhabdus luminescens* contains a toxin that has proven effective against a broad array of insect pests. It is a widely-dispersed, multiple strain bacterium that lives inside of and in symbiosis with soil-dwelling nematodes.

The discovery of a diverse new family of insect-killing bacteria has added importance since, in recent years, some insects have begun to exhibit resistance to the Bt toxin, raising fears that the biological pesticide may be losing its potency.

In concentrated doses, the newly-identified toxin can be used as a spray or fed directly to insects. The greatest potential application, however, lies in transferring the toxin-producing genes from the bacteria to crop plants. The researchers have identified, cloned and sequenced the genes responsible for the Photorhabdus toxin. Clones were indepen-

dently derived at DowElanco as well. The Photorhabdus toxin and the genes that produce it have been patented jointly by the Wisconsin and DowElanco scientists through the Wisconsin Alumni Research Foundation (WARF). The technology has been licensed to DowElanco.

The next step, already well underway, is to move those genes to any amenable crop plant. Bringing a product to the field, however, may still take anywhere from three to five years. (Source: *The AgBiotech Bulletin*, February 1998)

New tool to fight TB

A team of scientists at the Howard Hughes Medical Institute (HHMI) at the Albert Einstein college of Medicine (Bronx, NY) has discovered an efficient technique for creating mutations in *Mycobacterium tuberculosis (M. tb)*, the bacterium that causes the disease. Until the new development, *M. tb* resisted most traditional transposon mutagenesis methods.

The researchers used a novel type of vector called "conditioning replicating shuttle phasmids" that carry transposons inside cells. The mutation libraries generated provide information about the function of individual bacterial genes.

Microbiologist William R. Jacobs, Ph.D., immunologist Barry R. Bloom, Ph.D., and their colleagues worked for 10 years to construct special delivery vectors to transport transposons inside *M. tb* cells. Their now successful vector combines genetically engineered mycobacteriophages with an *E. coli* cosmid. This chimera replicates in *E. coli* as a plasmid and Mycobacteria as a phage, transferring DNA to both. Dr. Jacobs named the dual-purpose chimera a "shuttle phasmid".

More people die from TB than from malaria, diarrhea, AIDS and tropical diseases combined. Current treatment of TB requires taking at least two antibiotics, usually isoniazid and rifampicin, for a minimum of six months.

Because many people fail to complete the lengthy therapy, treatment failures are high and multidrug resistance is increasing. The World Health Organization reports that 10 per cent of new cases of TB are now resistant to at least one drug.

M. tb is a notoriously difficult microbe to study in the laboratory. Standard molecular techniques that researchers use to routinely damage DNA and generate mutants in other microbes fail in *M. tb*. Such mutants are extremely valuable in the study of disease pathogens, because they allow scientists to examine the effect of individual gene mutations on an organism's ability to survive or cause disease. Mutant analysis reveals new drug targets or potential vaccine candidates.

M. tb has an unusually tough, waxy, impermeable coat that makes it difficult to get gene-modifying agents inside cells or separate single cells to study their mutations. To grow a mutated *M. tb* colony for study takes three to four weeks, compared with just eight hours for cooperative bacteria like *E. coli*. *M. tb* also requires special containment facilities to handle them safely while they grow.

Dr. Jacobs collected phages of Mycobacteria from his backyard soil in the Bronx and isolated their genomes. He won the "prokaryotic lotto", he says, when he found a mutant phage that infected and transformed *M. tb* at a frequency seven orders of magnitude higher than its parent phage.

The researchers then developed conditional shuttle phasmids with mutations that prevented them from replicating at 37° C. The attached transposon carried by the shuttle phasmids contained a gene that made them resistant to the antibiotic kanamycin. The shuttle phasmids were mixed with *M. tb* cells and incubated at 37° C. At this non-permissive temperature, the phasmids entered the bacterial cells, and the transposons inserted themselves into the bacterial DNA. If the experiment had been done at a different temperature, the phasmid would have replicated and destroyed the cells.

Next, the phasmid/*M. tb* cell mixture was transferred to culture media containing kanamycin. Only cells that had undergone transposon mutagenesis and acquired the kanamycin-resistant gene will grow on this medium. Thousands of kanamycin-resistant mutants were recovered that showed random distribution of transposon insertions. With these methods, the researchers should be able to create mutations in virtually every gene of *M. tb*.

So far, the Howard Hughes researchers have collected at least 3,000 mutants. Although this method for creating mutants of *M. tb* is the best to date, "it's not so wonderful that people should think that the TB messiah has arrived", says Dr. Bloom. Once the mutants are made, it still takes a minimum of three weeks for *M.tb* to grow into colonies for analysis.

The new method of creating *M. tb* mutants attracted the attention of Glaxo Wellcome in Middlesex, UK. Drs. Jacobs and Bloom will become scientific collaborators with Action TB, a five-year-old research initiative aimed at finding new treatments for the disease. Action TB research will focus on moving drug targets into high-throughput screening, developing rational drug designs, understanding how TB evades detection by the immune system, and identifying vaccine candidates. Drugs used today to treat TB, such as isoniazid and rifampicin, were developed in the 1950s and 1960s and their cellular targets remain largely unknown. Using shuttle phasmids to generate mutants of *M. tb* provides an unprecedented opportunity to make rapid and substantial progress in understanding the pathogenesis and development of new therapeutics and vaccines for TB. (Source: *Genetic Engineering News*, 15 May 1998)

New variation on TB susceptibility

Researchers at the Wellcome Trust Centre for Human Genetics (Oxford University, UK) have identified for the first time a non-HLA genetic factor affecting susceptibility to tuberculosis in human populations. By typing a West-African population for known poly-morphisms in the gene for natural resistance associated with macrophage protein 1 (Nramp1)—a protein whose mouse homolog controls resistance to certain mycobacteria—Adrian Hill and his team found that four polymorphisms were significantly associated with tuberculosis. Subjects heterozygous for two of them, a single nucleotide change in intron 4 and a short deletion in the 3′ untranslated region (3′UTR), are at fourfold higher risk of developing the disease. Interestingly, the 3′UTR variant was present in 25 per cent of the West African population studied, much more prevalent than in Europeans. "It is important to stress that *NRAMP1*, despite being clearly involved in determining susceptibility to tuberculosis, is not the major genetic factor governing resistance in humans", states Hill, who led the study. He is currently attempting to confirm whether Nramp1 is a macrophage metal transporter protein, similar to a homologous protein, Nramp2. (Source: *Nature Biotechnology*, Vol. 16, May 1998)

Tuberculosis microbe sequenced

In June researchers reported obtaining the DNA sequence of the complete 4.4-Mb genome of *Mycobacterium tuberculosis*, the organism that causes tuberculosis. The

sequence is the first completed at The Wellcome Trust Pathogen Genome Unit at the Sanger Centre, UK.

An estimated 2.9 million people died from this chronic infectious disease in 1997, and concern is growing over new antibiotic-resistant strains that have emerged in recent years. According to a *Nature* online special report on the global tuberculosis epidemic (www.nature.com), about one in every three people in the world is infected with *M. tuberculosis*, and each has an estimated 10 per cent lifetime risk of progressing to clinical disease. Scientists hope that knowledge of the DNA sequence will provide clues to designing more effective therapeutic agents and vaccines.

The sequence is accessible from the Sanger Centre Web site (www.sanger.ac.uk/Projects/M_tuberculosis).

A tool is also available through the South African National Bioinformatics Institute for searching and extracting genome sequence and open reading frames from the genome of *M. tuberculosis* (ziggy.sanbi.ac.za/tbsearch.html). Searches also can be performed against incomplete *M. leprae* data. (Source: *Human Genome News 9 (3)*, July 1998)

Bug killer

Molecules that mimic DNA could be turned into a new family of antibiotics, say Danish scientists.

The molecules are based on peptide nucleic acid (PNA). In PNA, nucleotide bases like those in DNA and RNA are attached to a backbone of linked amino acids.

Researchers at the University of Copenhagen say they have now produced PNA molecules that have base sequences designed to stick to key parts of RNA in *Escherichia coli*. They hoped this would stop the bacteria from making proteins and kill them.

In laboratory tests, the researchers found that the PNA was just as good as the antibiotic tetracycline at killing the bacteria. (Source: *New Scientist*, 28 March 1998)

Electronic industry interested in copper-eating bacterium

A new bacterium which feeds on copper has been identified by researchers at the University of Abertay, Dundee, according to *Biotechnology Scotland*. A chemolithotroph which feeds on rocks and metals, the bacterium is special because it feeds on copper. It is anticipated that it will be useful to bioremediate copper pollution generated in the production of semiconductors.

Contact: Dr. Phil Collier, University of Abertay, Dundee, Scotland. Tel.: 44 0 1382 308000. (Source: *The AgBiotech Bulletin*, February 1998)

Drug resistance in hospitals traced to the farmyard

Farmers are the prime suspects in the creation of a human "superbug", according to genetic evidence linking a farmyard antibiotic to the emergence of a drug-resistant human pathogen. The discovery will fuel calls for a ban on the use of antibiotics to promote the growth of farm animals.

Bacteria called enterococci normally live harmlessly in the guts of people and animals, but can kill people with impaired immune systems. Increasingly, they are becoming resistant to drugs. In 1986, a strain that was resistant to vancomycin, an antibiotic used as a last resort, appeared in France. Similar superbugs soon emerged elsewhere in Europe, and from 1989 they spread through hospitals in the US.

At first, experts blamed the emergence of vancomycin-resistant enterococci (VRE) on the overuse of antibiotics in hospitals, but studies showing that pigs and poultry harboured VRE raised suspicions that perhaps they were originally food borne pathogens.

Evidence linking human VRE to farmyard antibiotics has been hard to find. But Henrik Wegener of the Danish Veterinary Laboratory in Copenhagen has revealed the best data yet.

Wegener has sequenced the gene responsible for vancomycin resistance in animal and human enterococci. Aside from the mutation that confers resistance, genes from some of the bacteria also carry a second mutation, where one letter of the genetic code, a G, is replaced with a T.

VRE samples from people contained both variants, bacteria from poultry in several countries were all of the T variant, while bacteria from pigs were all G. "This supports the idea that animals are the source of VRE in humans, whereas humans are an unlikely source of VRE for animals", says Wegener. "If that was the case, we would expect to see both in animals."

"Antimicrobial use in food production is a growing threat to human health", agrees David Heymann of the WHO's Division of Emerging and Other Communicable Diseases in Geneva. (Extracted from *New Scientist*, 21 March 1998)

Hot and muddled

Microbes that seek extreme heat do not seem to care how their genes are organized, say scientists who have completed the second DNA sequence from such a creature.

Unlike genes in most other organisms, genes that work together and even parts of the same gene can be widely separated in the genome of *Aquifex aeolicus*, a bacterium that grows in hot springs at up to 95° C. The same genetic disorganization is evident in the sequence of another heat lover, *Methanococcus jannaschii*.

Team leader Ronald Swanson of Diversa Corporation in San Diego, California, says these unrelated microbes can tolerate such genetic chaos because they are adapted to grow under extreme, but very consistent conditions. (Source: *New Scientist*, 28 March 1998)

Protein from Y. pestis could bring pain relief

Bubonic plague—one of history's most notorious killers—could hold the key to easing the pain of millions of people suffering from arthritis and other inflammatory diseases. Now researchers at Porton Down, the Ministry of Defence site in Wiltshire, have found that a key protein from the bacterium *Yersinia pestis* can damp down inflammation.

When *Y. pestis* enters the body the bacteria are transported to the lymph nodes but they escape and cause disease. They establish themselves by inhibiting the inflammatory responses that would otherwise kill them.

Hill's team suspects that the V antigen plays a key role in this process. The protein increases interleukin 10, a signalling molecule known as a cytokine which usually damps down inflammation. It does this by suppressing production of an inflammatory cytokine that is called tumour necrosis factor alpha.

Work by researchers at the US Army Medical Research Institute also established that V antigen inhibits the migration to sites of infection of white blood cells called neutrophils, which destroy invading organisms. Mice dosed with Vantigen were able to tolerate injections of lipopolysaccharide, a substance that normally produces instant and violent immunological responses.

Taken together, says Hill, these findings all suggest that the V antigen could dampen down bouts of unwanted

inflammation that occur in patients with autoimmune diseases, such as arthritis.

Hill is confident that the team at Porton Down can isolate the fragments of the protein most vital for immunosuppression—drugs with small molecules are likely to be easier to produce and administer than the complete protein. (Source: *New Scientist*, 9 May 1998)

Staphylococci get the RAP

By blocking a bacterial signalling pathway, a vaccine developed by researchers at the University of California, Davis School of Medicine may offer a new treatment for patients who become infected with antibiotic-resistant bacteria. *Staphylococcus aureus* can cause life-threatening infections when it enters a wound. During infection, it secretes a protein, called RAP, that signals a shift from colonization to invasive growth. The proteases attack the surrounding tissue and can cripple the host's immune system before it can respond. The research team reasoned that blocking toxin production would allow the immune system to eliminate the invading bacteria. By injecting rats with purified RAP, they were able to induce the production of antibodies which prevent the protein from sending its signal. The rats' immune systems then happily destroy the microbes. The approach has been licensed by Panorama Research (Mountain View, CA), which plans to develop the vaccine for use in humans. (Source: *Nature Biotechnology*, Vol. 16, June 1998)

Mean microbes

Tiny bacteria may cause kidney stones, a scientist in Finland claims. Olavi Kajander of Kuopio University says these enigmatic "nanobacteria" surround themselves with mineral shells that could cause harmful calcium deposits in the body.

Kajander found the bacteria, which are less than 0.1 micrometres long, in the blood of humans and cows. He says they are genetically similar to other established types of bacteria such as *Brucella* and *Bartonella*.

Kajander and his colleague Neva Çiftçiglu say the bacteria erect mineral coatings, and because the mineral coatings are similar to the aggregates found in kidney stones, he suspected they might be the cause of the stones. Sure enough, he found that cells infected with the nanobacteria developed mineral deposits both inside and outside. Tests on 30 human kidney stones showed they all contained the bacteria.

In a commentary on the research, Dennis Carson of the University of California at San Diego says that these bacteria or their close relatives may figure in a host of other human diseases. These include heart disease, some tumours and dementia due to abnormal calcium deposition in the brain.

Killing these bacteria would take some time because they are protected by their mineral coats, but Kajander suggests that long courses of the antibiotic tetracycline might eradicate them. (Extracted from *New Scientist*, 11 July 1998)

Lactic acid fights killer E. coli

Biofermin Pharmaceutical, a specialized maker of medicine for intestinal disorders and lactic acid drugs, has verified through outsourced testing that its triple lactic acid drug is effective in fighting *E. coli* O-157 infection even if taken prior to the infection as a preventative measure.

Experiments using mice were conducted by a research group affiliated with the medical department of Tokai University. The experiments included a group of mice infected with the *E. coli* bacteria orally but not given the lactic acid treatment, and a group of mice given the triple lactic acid agent prior to infection with the bacterium. The death rate after a period of 14 days was 79 per cent for the group not given the lactic acid treatment versus 0 per cent for the group given the treatment. In addition, compared to the non-lactic acid group, the O-157 bacterium concentration in the stool of the mice given the treatment decreased markedly on the third, seventh and fourteenth days. (Source: *McGraw Hill's Biotechnology Newswatch*, 10 July 1998)

The bug issue

A common anti-microbial agent valued for the indiscriminate way it kills bugs is not as unselective as once thought, US researchers have discovered. The findings raise the prospect of new disinfectant-resistant bacteria and fungi.

The agent, called triclosan, is used as a "biocide" in soaps, toothpastes, fabrics and plastics. Researchers once believed it worked by chemically dissolving the cell walls of bacteria and fungi. This attack would have been so unspecific that resistant strains would never have evolved.

But a research team based at Tufts University in Boston, Massachusetts has isolated five strains of *Escherichia coli* that can withstand triclosan.

The team found that resistant strains carry mutations in a gene called *fabI*, which encodes an enzyme involved in building the cell wall of *E. coli*. This suggests that triclosan specifically attacks this enzyme, the researchers say. Blocking *fabI* would produce an effect the same as dissolving the cell wall.

If resistant strains emerged, operating theatres and other sterile environments would find it difficult to eliminate bacteria. However, triclosan resistance has yet to be seen outside the laboratory.

The study has implications for other "broad spectrum" biocides. (Source: *Chemistry and Industry*, 17 August 1998)

Superbug gives up its secret

Antibiotics are increasingly useless against certain strains of gut bacteria called enterococci that have evolved resistance to them. In some people whose immune systems are weakened, such as patients having surgery to their heart valves, such superbugs can be life threatening. Now researchers in New York have discovered the structure of the enzyme that makes some of these bacteria resistant to the antibiotics. The finding opens the way to designing drugs to overcome them.

The enzyme makes bacteria resistant to gentamicin, one of a group of antibiotics called aminoglycosides. These antibiotics normally bind to specific sites in bacterial ribosomes, the structures where proteins are made, and disrupt their function, thus disabling the bacteria and curing infection.

Antibiotic-resistant bacteria make an enzyme that binds to the aminoglycosides and thwarts their action. Researchers at Rockefeller University in New York used X-ray crystallography to map the structure of the enzyme, called aminoglycoside N-acetyltransferase. They say its shape resembles a right hand grasping a cylinder. The gap is where the enzyme seems to bind to gentamicin. (Source: *New Scientist*, 29 August 1998)

Could designer antibiotics hit bugs where it hurts?

Single genes that infectious bacteria depend on for their survival have come to light. The researchers who made the

discoveries say their technique could lead to a new generation of antibiotics.

Resistance to antibiotics is increasing among our microbiological foes. Existing antibiotics can be designed to outwit the bacteria, but they soon develop resistance to the new line of attack.

One alternative is to find genes that are essential for the survival of bacteria but not humans, and to target these genes with new drugs. Hannes Loferer of Genome Pharmaceuticals in Munich and his colleagues reasoned that genes that are common to many species of bacteria would be likely candidates. Biologists have long suspected that genes that are widely conserved are vital for life.

To date, the entire genomes of 14 species of bacteria have been sequenced, including that of *Mycoplasma genitalium*, one of the simplest bacteria, which has only about 600 genes. Loferer's team compared *M. genitalium's* DNA sequence with that of *Escherichia coli* and found that they have 26 genes in common.

To identify which of these genes are essential to *E. coli*, the researchers engineered 26 mutant strains that each lacked one of the genes. They then tested the mutants to see if they could survive. The team say that of the 26 genes, six were found to be essential for survival. Four of these were not essential to yeast, and may well be non-essential to humans, suggesting that they may be good targets for future antibiotics. (Source: *New Scientist*, 5 September 1998)

Tiny timekeepers

Some bacteria have biological clocks similar to those of flies and people. An international team of researchers has identified three clock genes in a group of bacteria that rely on the Sun for energy.

Photosynthetic microbes called cyanobacteria are the most primitive organisms known to have a daily cycle of gene activity. Even without light, they continue to switch on genes for photosynthesis during the day.

Takao Kondo of Nagoya University in Japan and colleagues have isolated the genes responsible and dubbed them *kaiA, B* and *C* (*kai* is Japanese for cycle). Although the gene sequences are very different from those of genes that regulate daily rhythms in animals, Kondo believes the way the clock works is similar. One gene, *kaiA*, turns on the other two, which then turn themselves off again. Kondo says the next step is to work out how the cycle is synchronized with day length. (Source: *New Scientist*, 12 September 1998)

Set in stone

The formation of sedimentary rocks is usually an excruciatingly slow process, taking millions of years. But the two bacteria, discovered by a research team led by Max Coleman, a sedimentologist at the University of Reading, do the job in as little as six months.

While digging survey trenches in Norfolk salt marshes, Coleman noticed strange stony nodules buried in the mud. Some were as large as footballs.

Coleman has established that a pair of bacteria join forces to create the nodules. The first, a species of *Desulphobacter*, gets its energy by consuming sulphates in seawater and reducing them to hydrogen sulphide. The second, *Desulphovibrio desulphuricans*, can also perform the same chemical reaction. But when its environment contains too much hydrogen sulphide, it switches to reducing iron compounds, converting Fe^{3+} ions to Fe^{2+}. The latter react with the hydrogen sulphide and other salts to create stony deposits of iron sulphide and iron carbonate.

These reactions do not seem to run in reverse, but if the nodules are exposed to air, the iron at the surface can be oxidized once more, to form a layer of rust.

Coleman believes the nodules could be a rich source of fossils. Because the rock forms so quickly, he says, dead organisms might be preserved before they can rot. (Source: *New Scientist*, 19 September 1998)

New enzyme structure

Imagine solving a 30,000-piece three-dimensional puzzle when you do not know what it is supposed to look like and when many of the pieces are identical. That is essentially what scientists at the National Institute of Standards and Technology and the Center for Advanced Research in biotechnology did recently when they solved the three-dimensional structure of an enzyme called threonine deaminase, a large biological molecule produced by the bacteria *Escherichia coli*. The enzyme structure has long intrigued scientists as it has a switch on one end for turning itself on or off.

Since the enzyme helps produce an essential amino acid for *E. coli* bacteria, pharmaceutical researchers now can use its structure as a target for developing new antibiotic drugs. Because plants also use the enzyme, inhibiting it may offer a new strategy for weed control. Plastics manufacturers are interested in the enzyme because it produces a compound used to make biodegradable plastics. Modifications in the enzyme could improve efficiency in biodegradable plastic production.

To solve the structure, researchers first produced crystals of the enzyme, and then computed a three-dimensional map of the structure from data they obtained by illuminating the crystals with X-rays. Next, they painstakingly matched protein building blocks to the map until the structure was solved.

Media contact: Linda Joy, Tel.: (301) 975-4403 (Source: *Tech Beat*, September 1998)

Research instrumentation

Molecular cages catch genes in the act

A technique developed by chemists in California generates three-dimensional images that can be used to track a gene's activity through the stages of a growing embryo.

Compounds of metal ions such as gadolinium can increase the brightness of the magnetic resonance imaging (MRI) signal produced by neighbouring water molecules. Such "contrast agents" light up wherever water is present. Thomas Meade and his colleagues at the California Institute of Technology in Pasadena have made "smart" contrast agents, which cause strong signals only when a particular gene has been activated.

To do this they designed a molecule that holds gadolinium in a cage, denying water molecules access to the metal and so turning off the MRI signal. The cage can be designed so that when a particular enzyme is present, it clips off the cage's roof, allowing water in and turning the signal on.

Meade and his team tested their idea by introducing messenger RNA for a bacterial enzyme into one cell of a four-cell embryo of an African clawed frog, *Xenopus laevis*. When the embryo had divided to form 16 cells, the researchers injected the caged contrast agent into some of the cells. Only the glowing descendants of the original cells showed up on an MRI scan.

Meade says his MRI contrast agent can be adjusted to respond to different enzymes, so the technique could have clinical uses, such as tracking the activity of protease produced by HIV. (Source: *New Scientist*, 12 September 1998)

Golden genes

Chemists have found a new way to spot genetic mutations by using gold.

When ground into spheres 13 nanometres across, electrons in the particles rush back and forth when illuminated, making a solution of them appear red. By changing the spacing of the particles the colour of the solution is also changed.

Chad Mirkin and his colleagues at Northwestern University in Evanston, Illinois, attached gold particles to DNA segments. The segments initially appeared red, but when the team added complementary DNA, the gold-DNA bound to the new strands and clumped together, turning the solution purple.

If there is a mutation in the added DNA, giving an imperfect match, the complexes fall apart when heated and the solution turns red again. (Source: *New Scientist*, 28 February 1998)

DNA chips to the rescue

Analysis of interacting clones from a yeast two-hybrid screen can now be hastened using DNA chip technology. In two separate experiments, Ron Davis and colleagues at the Stanford University School of Medicine (Stanford, CA), in collaboration with Affymeterix (Santa Clara, CA) and the Institute Pasteur (Paris), have screened a yeast expression library for proteins interacting with Ymr117c and Ymrl38w. With the exception of three loci, the array correctly identified all the genes present in clones identified using two-hybrid screens. According to the authors, the remaining loci were not detected because they were either not represented on the DNA chip or recalcitrant to PCR amplification. "The results obtained using the array screening were comparable to those obtained by dideoxy sequencing", says Ray Cho of Stanford University, an author on the paper. "The power in coupling the two technologies lies in the fact that pools of interacting cones from thousands of two-hybrid screens can now be analysed in a very short space of time, bypassing the dideoxy sequencing step for each cone, adds Affymetrix's Lockhart." (Source: *Nature Biotechnology*, Vol. 16, June 1998)

Partnership formed to create new biochips

Packard Instrument Co. (Meriden, CT), Motorola, Inc. (Schaumberg, IL), Argonne National Laboratory (Argonne, IL) and the Engelhardt Institute of Molecular Biology (Moscow, Russia) formed a partnership to develop an advanced biochip system that may significantly accelerate genetic analysis.

The greatest impact would be in medical diagnosis, with markets potentially in the low billions of dollars. The system is also expected to hasten drug discovery, improve bioremediation and contribute to crop biotechnology.

By combining Motorola's mass production capabilities, Packard's core competencies in bioanalytical equipment and reagents and Argonne and Engelhardt intellectual property and expertise, the partners believe they can make the biochip technology accessible to a broad range of markets for a wide range of applications.

The essential feature of the biochip is a platform the size of a microscope slide containing 10,000 tiny polyacrylamide gel tabs, each of which can be used for analysis of a separate target piece of DNA, RNA or protein.

Known compounds are placed in the gel tabs, and compounds being analysed are dispensed into the gel tabs through tiny glass capillaries, about 1.5" long, but with a tip that tapers to 75 microns at the opening, to mate with the 100 x 100 micron gel tabs. A tiny cylinder (a piezoelectric element) surrounds the capillary. "Every time you apply an electrical pulse to that element, it squeezes the capillary 1,000 times a second", says Van Cauter of Packard. Each capillary can shoot as little as 200 picolitres into the gel baths.

The dispensing system, called the BioChip Arrayer, has four capillaries, but the second generation, expected next year, will have eight, and the third generation, scheduled for 2000, will have 96. The system will identify mutations, or polymorphisms, through hybridization of known genetic material in the gel tabs with samples dispensed by the BioChip Arrayer. Proprietary fluorophore tags will be used to detect hybridization, and Van Cauter says it will be sensitive enough to detect single-base differences between nucleic acids.

In the case of a chip to detect genetic disease, as many as 1,000 markers might be involved, and as many gel tabs would contain short oligonucleotides containing the defective sequences, says Van Cauter. "You would take a blood sample from the patient, extract, purify and amplify it, and then apply it to the chip." A strong signal from the fluorophores in one of the wells would indicate that a mutation exists in the patient's DNA.

"Once biochip technology becomes widely available at low cost, medical and biological researchers will be able to identify in minutes mutated genes that could lead to later medical problems, such as cancer, multiple sclerosis or Alzheimer's", says an Argonne spokesperson. "By using less than a drop of solution, doctors will be able to predict drug efficacy, to diagnose drug resistance to treatment of diseases like AIDS and TB and to make on-the-spot identification of specific bacteria, viruses, and other micro-organisms."

Similarly, as researchers discover genetic characteristics that predict whether a specific drug can treat a specific individual, this technology could be used to help doctors prescribe therapeutics more effectively.

Gene regulation and drug discovery experiments could be done quickly and easily.

Biochips could be used as detectors of microbial or chemical pollution. They could also be employed to identify genes for detoxifying enzymes, which could then be engineered into bacteria for bioremediation. Also, genes for disease resistance and the like could be identified in plants and engineered into crop plants. Genes for harmful substances in foods, such as fat, could be identified and modified to reduce the fat content of meat.

While not part of current research at the collaborating institutions, forensic crime detection could also benefit. The biochips "make possible for more accurate and sensitive tests to compare, for example, a suspect's DNA with that found at a crime scene", according to researchers at Argonne. (Source: *Genetic Engineering News*, August 1998)

High-throughput sequencing method aims at cancer markers

Doctors hope to discover more distinctive genetic markers for cancers using a new technique similar in concept to the high-throughput sequencing that helped revolutionize genome research.

The new genetic technique can analyse hundreds of tissue samples at the same time. It promises to give faster and more accurate assessments of cancer tumours, according to doctors at the National Human Genome Research Institute in Bethesda, MD.

Traditionally, cancer tissue specimens have been analysed one at a time, making it difficult to compare and contrast nuances in different types of cancer.

The new technique lets doctors screen hundreds of tumour specimens from patients in different stages of disease, making it easier to tell the distinguishing genetic features of each type of cancer.

Dr. Olli-P Kallioniemi, section head at the Laboratory of Cancer Genetics, National Human Genome Research Institute and his colleagues tested the new technique by analysing 645 breast cancer tissue samples in less than two hours.

They were able to distinguish sub-groups of tumours within the samples by detecting six different cancer-associated genes.

The system can analyse up to 1,000 samples at a time. In can also be adapted to study tissues associated with other diseases. The researchers have studied 500 prostate cancer tumours as well. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 6 July 1998)

Simplified method for dendrimer synthesis

A simplified technique has been established to synthesize dendrimers, spherical polymer compounds, as a part of the Technology for Novel High-Functional Materials project, implemented through the Industrial Science and Technology Frontier Program of the Agency of Industrial Science and Technology of Japan.

The dendrimer, due to its characteristic spherical shape and the arrangement of numerous functional groups on its molecular surface, is anticipated to be a new functional material for various applications such as pharmaceuticals, electronic materials, chemistry and micromachines. However, its synthesis requires an AB2 type monomer as the starting material which is linked to a central nucleus by sequential bonding (divergent method). In another method, an external nucleus is first formed from AB2 molecule, and then is bonded to the central nucleus (convergent method). However both these methods require protection of active groups with special protecting agents and repeated multistep reactions including deprotection reactions. Such a complicated multi-step synthesis was a major obstacle in the mass production of dendrimers and there has been no example of its commercialization.

The research team investigated a method to synthesize dendrimers with the central nucleus consisting of multifunctional compounds. The central nucleus is formed of calixarenes, which are large cyclic compounds with 8-16 hydroxyl groups as reactive groups. Using these calixarene compounds, dendrimers consisting of benzyl ether units were synthesized. As a result, it became possible to obtain dendrimers with molecular weights of as high as 9,300 even with second generation versions. The dendrimers obtained feature a high surface functional group density, can be incorporated with the alkali soluble property, and are expected to be applicable as functional materials.

Further details from National Institute of Materials and Chemical Research, AIST, 1-1, Higashi, Tsukuba, Ibaraki City Pref. 305-8565. Tel.: +81-298-54-6348; Fax: +81-298-54-6327. (Source: *JETRO*, February 1998)

General

Anticipated benefits of genome research

Predictions of biology as "the science of the 21st century" have been made by observers as diverse as Microsoft Chairman Bill Gates and US President Bill Clinton. Already revolutionizing biology, genome research has spawned a burgeoning biotechnology industry and is providing a vital thrust to the increasing productivity and pervasiveness of the life sciences.

Technology and resources promoted by the Human Genome Project already have had profound impacts on biomedical research and promise to revolutionize biological research and clinical medicine. Increasingly detailed genome maps have aided researchers seeking genes associated with dozens of genetic conditions, including myotonic dystrophy, fragile X syndrome, neurofibromatosis types 1 and 2, inherited colon cancer, Alzheimer's disease, and familial breast cancer.

Current and potential applications of genome research will address national needs in molecular medicine, waste control and environmental cleanup, biotechnology, energy sources, and risk assessment.

On the horizon is a new era of molecular medicine characterized less by treating symptoms and more by looking to the most fundamental causes of disease. Rapid and more specific diagnostic tests will make possible earlier treatment of countless maladies. Medical researchers also will be able to devise novel therapeutic regimens based on new classes of drugs, immunotherapy techniques, avoidance of environmental conditions that may trigger disease, and possible augmentation or even replacement of defective genes through gene therapy.

In 1994, taking advantage of new capabilities developed by the genome project, DOE formulated the Microbial Genome Initiative to sequence the genomes of bacteria useful in the areas of energy production, environmental remediation, toxic waste reduction, and industrial processing. As a result of this initiative, six microbes that live under extreme conditions of temperature and pressure had been sequenced completely as of August 1997. Structural studies are under way to learn what is unique about the proteins of these organisms—the ultimate aim being to use the microbes and their enzymes for such practical purposes as waste control and environmental cleanup.

The potential for commercial development presents US industry with a wealth of opportunities. Sales of biotechnology products are projected to exceed $20 billion by the year 2000. The project already has stimulated significant investment by large corporations and prompted the creation of new biotechnology companies hoping to capitalize on the far-reaching implications of its research.

Biotechnology, significantly fueled by insights reaped from the genome project, will play a significant role in improving the use of fossil-based resources. Increased energy demands, projected over the next 50 years, require strategies to circumvent the many problems associated with today's dominant energy technologies. Biotechnology promises to help address these needs by providing cleaner means for the bioconversion of raw materials to refined products. In addition, there is the possibility of developing entirely new biomass-based energy sources. Having the genomic sequence of the methane-producing micro-organism *Methanococcus jannaschii*, for example, will enable researchers to explore the

process of methanogenesis in more detail and could lead to cheaper production of fuel-grade methane.

Understanding the human genome will have an enormous impact on the ability to assess risks posed to individuals by environmental exposure to toxic agents. Scientists know that genetic differences make some people more susceptible—and other more resistant—to such agents. Far more work must be done to determine the genetic basis of such variability. This knowledge will directly address DOE's long-term mission to understand the effects of low-level exposures to radiation and other energy-related agents, especially in terms of cancer risk. [Reprinted from the DOE Human Genome Program Report] (Source: *Human Genome News 9 (1-2)*, January 1998)

Genomes of major pathogens will be made public

The Wellcome Trust frustrated by drugs companies who keep their research results secret, the world's largest medical research charity has decided to provide £205 million to sequence the genomes of several micro-organisms that cause important human diseases.

Deciphering the genetic code of pathogens should make it easier to find drugs that attack them. But obtaining the complete DNA sequence of even unicellular organisms can be a long and tedious process. Companies hardly ever release such sequencing information for fear that others will exploit it before they do. As a result, some bacteria have been sequenced several times over, but the information is still secret.

Beowulf Genomics, a non-profit organization set up by the Wellcome Trust, has already commissioned the Sanger Centre in Cambridge to sequence the genome of *Campylobacter jejuni*, which causes food poisoning. This should be finished by the end of 1998. Among other micro-organisms on Beowulf's hit list are those that cause listeria, whooping cough, plague and thrush. The sequences will all be posted on the Web.

The Wellcome Trust's initiative follows last year's decision by The Institute for Genomic Research in Rockville, MD, to break from its commercial partner and release it sequences of pathogenic micro-organisms. (Source: *New Scientist*, 14 February 1998)

Genome project goes into overdrive

A private company has announced plans to sequence the human genome by 2001, four years sooner than the target date set by the publicly funded Human Genome Project.

All the data will be made freely available to researchers. "We decided it would be morally wrong to keep the data secret", says Craig Venter, president of Celera Genomics Corp. and founder of The Institute for Genomic Research (TIGR) in Rockville, MD.

Formed by TIGR and Perkin-Elmer, a scientific equipment manufacturer in Norwalk, CT, the new company will use powerful DNA sequencing machines to read the 30 billion bases in human DNA within three years. Venter estimates that sequencing the genome will cost around $200 million.

The news may prompt the US Congress to reconsider its budget of $3 billion for the Human Genome Project, half of which has already been spent, with 97 per cent of the genome still to be sequenced. Venter says that the new company is keen to collaborate with its public counterparts, and Francis Collins, head of the genome project at the National Institutes of Health near Washington, D.C., says he welcomes the new initiative.

The new company hopes to make money by identifying single-nucleotide polymorphisms, subtle variations in the same gene that predispose particular individuals to disease, and dictate which medicines will work for them. This information will be sold to pharmaceutical companies. (Source: *New Scientist*, 16 May 1998)

NIH to produce a "working draft" of the genome by 2001

In August, Incyte Pharmaceuticals Inc. of Palo Alto, CA, joined the race. It said it was going after the entire human genome too, aiming to get just the genes in two years. Now, the US National Human Genome Research Institute (NHGRI) has unveiled a five-year plan that promises to produce a "working draft" of the human genome—including highly accurate sequences of most of the protein-coding regions—by 2001. The plan also promises to yield a polished, gold-standard version of the entire genome by 2003, two years ahead of the old schedule. If successful, this scheme will not only speed up the pace at government-funded labs but also, according to some of NHGRI's advisers, release data so rapidly that companies such as Perkin-Elmer and Incyte may not be able to get exclusive rights to all the DNA they hoped to patent. (Extracted from *Science*, Vol. 281, 18 September 1998)

Medicine based on genetics will transform healthcare

If you go to your surgery twenty years from now complaining of rheumatism, your doctor may well check out the relevant section of your personal genome CD-ROM rather than reach straight for the prescription pad. Any medicines you are eventually given will be designed specifically for you. The days when everyone suffering from the same disease took the same drug will be long gone.

Pharmaceuticals companies are already preparing for a healthcare revolution based on genomics. Its foundations are being built by the Human Genome Project, which will have mapped and sequenced tens of thousands of human genes by 2007. The project, which involves scientists in more than 50 countries, has the potential to change medicine like no previous scientific advance. As the DNA sequence of every human gene is revealed, we will learn what each one does, how genes vary from person to person and how we might manipulate them with new drugs to beat disease.

But these advances will not come cheap. The drugs companies are facing a massive investment in the task of putting each sequenced gene under the spotlight. They need to discover its role and the protein it makes, then check its potential as a drug target. The huge cost of this research is causing massive upheavals in the industry.

Hunger for research funds is not the only driving force behind the changes, says Juan Enriquez, a Harvard economist specializing in biomedical research policy. Companies fear being locked out by their competitors' patents and licensing deals. One way to avoid this, he says, is to own your competitors.

For patients, a major benefit of genomics research will be more effective treatments. But the biggest change, and the most significant for health spending, will come from radically improved preventive medicine. Sequencing the human genome will give doctors and scientists powerful tools for assessing a particular patient's risk of certain diseases. Companies will be able to develop more effective drugs by expressing specific genes in cell cultures and working out the structure of the proteins they produce. This could result in earlier detection and treatment for Alzheimer's, cancer and

diabetes. It will also provide new opportunities for DNA vaccines, which are longer-lasting, more effective and easier to make than conventional protein vaccines.

But success is not guaranteed. Sceptics fear that the interaction between our genes and drugs could turn out to be more complex than imagined. Neutralizing a gene in order to inhibit a harmful biochemical process could affect other, unsuspected beneficial processes.

The sums required for medical genetics research far exceed what most drugs companies can currently afford. Even the biggest—Merck in the US and Glaxo Wellcome—are dwarfed by the top oil companies and motor manufacturers. Perhaps most significantly, the world pharmaceuticals market is fragmented, with the industry's two biggest players only commanding about 5 per cent each.

So drugs companies need to grow. The result has been a frenzy of alliances, mergers and takeovers. By 1996 a series of mergers, including that of the Swiss-based Ciba and Sandoz to create Novartis, had pushed it down to tenth place.

The effects of the new medicine are being felt beyond the boardrooms of pharmaceuticals groups. Funding of genetic research in universities has rocketed, with state grants being dwarfed by money from industry. In the US, Duke University is launching its own biotech company. Stanford and Cornell are restructuring their genetics departments to focus on genomics. It is also likely that some countries will reap the benefits more successfully than others. Analysts point out that new biotechnology firms do not necessarily result in new advances: the emergence of 1,400 such companies in the US has resulted in just 50 successful drugs. And first of all, companies must persuade the public that the new techniques are ethical and safe. (Extracted from *New Scientist*, 21 March 1998)

Foetuses destined for abortion may be used to test gene therapy

Controversial plans to treat unborn children with gene therapy have been given an even more contentious twist. Under a proposal presented to a US government panel in September 1998, this therapy would initially be tested on foetuses destined for abortion.

Earlier this year, French Anderson of the University of Southern California in Los Angeles announced that he was seeking approval for foetal gene therapy. He is still developing the techniques and will not be ready to start trials for at least three years.

Anderson pioneered human gene therapy in 1990, when he treated children with a hereditary disorder called severe combined immune deficiency (SCID), caused by the lack of an enzyme vital for the development of the immune system. At a meeting of the National Institutes of Health Recombinant DNA Advisory Committee (RAC) on 24 September, Anderson described how foetuses with SCID would be given healthy copies of the gene for the enzyme. He also outlined plans for *in utero* treatment of an inherited blood disease called alpha-thalassaemia, caused by a defect in the gene for part of the oxygen-carrying molecule haemoglobin.

One concern is that a foetus's small size means the therapeutic gene has a greater chance of invading reproductive tissue and introducing genetic changes that will be passed down the generations.

But the committee had particular concerns about alpha-thalassaemia, says Claudia Mickleson, biosafety officer at the Massachusetts Institute of Technology and chair of the RAC. We each carry four copies of the gene that is defective in people with alpha-thalassaemia. In the worst cases all four copies are damaged and foetuses die in the womb or shortly after birth. The mother can also develop a life-threatening condition called pre-eclampsia, which involves high blood pressure and fluid retention.

Anderson is now suggesting his team could get round this dilemma by asking women who had already decided to abort their foetus to take part in the first trial. Some RAC members agree that this avoids the ethical problems of a partial cure but, as Anderson admits, it has difficulties of its own. After aborting the foetus, the researchers might discover that they had managed to cure the condition. The only solace, he says, is that the family would then be able to attempt another pregnancy knowing there was a treatment if the next foetus inherited the disorder. (Source: *New Scientist*, 10 October 1998)

Memory protein isolated

Researchers from the National Center of Neurology and Psychiatry, National Institute of Neuroscience (NCNP) and Nippon Suisan have discovered a new compound that increases the activity of a glutamic acid receptor protein that appears to be related to the process of memory.

The receptor regulates the intake of calcium ions on the surface of nerve cells. As calcium ions increase, the nerve cells become more active.

The compound discovered by the research group is similar to, but about 100 times more powerful than a brain metabolism agent based on a marine product extract to which Nippon Suisan holds a patent.

It is thought that diminished memory function witnessed in dementia patients may be the result of the reduced function of glutamic acid receptors.

The scientific team hopes to continue research based on animal experiments with the aim of developing pharmaceutical products and reagents in the future. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 March 1998)

Is there genuine hope of treating malaria at last?

The parasite that causes malaria has let slip clues to how resistant strains evade the drug chloroquine. The results have already led to tests of a new drug that may kill off all strains, chloroquine resistant or not.

Previous research had shown that chloroquine binds to a transporter molecule in the membranes of bacteria. Michael Lanzer and his colleagues at the University of Würzburg suspected that the malaria parasite, plasmodium, uses a similar membrane transporter to absorb the drug.

The team studied one such membrane molecule, an ion transporter that pumps sodium and hydrogen ions in and out of the cells. Their experiments showed that chloroquine triggers the transport mechanism, which shunts the drug into the cell.

The researchers found that this ion transporter is less active in plasmodium strains that are resistant to chloroquine. While the drug normally activates its own uptake, in resistant plasmodium strains it knocks on doors in vain. The findings fit with the discovery of a gene whose mutation could be behind chloroquine resistance. Lanzer believes the gene codes for the membrane transporter he studied.

The findings suggest a new way of treating malaria. Although the ion transporter normally helps chloroquine to get inside the parasite and kill it, blocking the transporter completely should make the parasite unable to regulate ion exchange, and it would die.

In collaboration with Lanzer's group, pharmaceuticals giant Hoechst Marion Roussel has already started trials of drugs that inhibit the ion transporters. Lanzer says their early results have shown that the drugs kill both chloroquine-sensitive and chloroquine-resistant plasmodium strains. (Source: *New Scientist*, 28 February 1998)

Salivary glands could be used in gene therapy

Salivary glands can be transformed into drug factories using gene therapy, say researchers in the US. They hope one day to replace injections for conditions like diabetes and haemophilia with an engineered gland that secretes therapeutic proteins into the blood and digestive tract.

Salivary glands can produce 50 grams of digestive enzymes each day, and while most of this output goes into saliva and is swallowed, some ends up in the bloodstream. Not only can salivary glands produce lots of protein, they are also encapsulated, so new genes put into the gland's duct cannot escape into the rest of the body. These two qualities make the glands an ideal spot for gene therapy for diseases such as diabetes and haemophilia, which require treatment with daily doses of insulin and blood clotting factors respectively.

Bruce Baum of the US National Institute of Dental Research near Washington, DC and his colleagues recently used a modified cold virus to insert a human growth hormone gene into the salivary glands of rats. They report that serum levels of the growth hormone in the rats rose to 16 nanograms per millilitre—three times the therapeutic level necessary in a human. These levels were substantially higher than those achieved when Baum injected the same virus into rat muscle, a popular site for gene therapy.

Baum says that a cold virus is not the best way to deliver genes, because it provokes an immune response. The researchers have only been able to get a gene introduced into the salivary gland to produce its protein for around a week. But they did manage to get therapeutic levels of a functional protein into the blood, which proves that their approach is feasible. It could become clinically useful in the next few years as researchers develop more efficient and longer-lasting ways to deliver genes. (Source: *New Scientist*, 16 May 1998)

Suicidal brain cells can be saved

The brain can be permanently injured when its oxygen supply is temporarily cut off by incidents such as strokes or near drowning. A way has now been found to reduce the damage, even hours after the loss of oxygen.

Brain cells do not die as soon as they are deprived of oxygen. Instead, biochemical events are triggered that ultimately lead to their demise up to 12 hours later. In some cases, oxygen starvation sets off genetically programmed cell suicide, or apoptosis.

At a crucial stage in apoptosis the cell dispatches enzymes called caspases to shred all cellular proteins. David Holtzman and his colleagues at Washington University in St. Louis tried to save cells destined to die by blocking the action of caspases with a substance called BAF.

Holtzman's team placed rat pups in chambers with less than half the normal amount of oxygen for two hours, and then treated some of the rats with BAF. Rats that did not receive BAF lost about half the tissue in their cortex and hippocampus, but rats that got an injection of BAF up to three hours after emerging from the chamber lost no more than 20 per cent. The researchers hope to begin human trials of similar drugs in a few years. (Source: *New Scientist*, 9 May 1998)

Shaping up

A new technique for predicting the functions of proteins may be a godsend for scientists trying to make sense of human genome maps due to be completed early next century.

A gene's DNA sequence reveals which amino acids will appear in the protein the gene encodes. To guess a new protein's function, scientists often compare its amino acids with those of familiar proteins. If two proteins share much of their sequences, the chances are they do the same job.

But this takes no account of the protein's shape, so the predictions are often wrong.

Jacquelyn Fetrow of the Scripps Research Institute in La Jolla, CA and her colleagues Jeffrey Skiolnick and Adam Godzik can now forecast a protein's function more accurately by guessing its 3D structure. They use a computer program that predicts which of 300 known protein shapes the unknown protein can fold into, and assume the actual structure is the most stable of these. They then pick out amino acids that play important roles in other proteins, and decide where on this structure they lie. This reveals the active sites, giving a good indication of what the proteins do.

The team tested the method on proteins that the *Escherichia coli* genome encodes. They identified the 10 *E. coli* proteins that belong to a class of enzymes called thioldisulphide oxidoreductases. (Source: *New Scientist*, 17 October 1998)

Seeds of life

DNA could survive without water in the vacuum of space for hundreds of thousands of years, researchers in California have suggested. Their discovery will encourage those who believe life may have originated in space.

Water plays an important role in keeping proteins folded into three-dimensional structures. But scientists were unsure how DNA would fare without water, in a vacuum. To test this, Evan Williams and his colleagues from the University of California at Berkeley placed DNA in evacuated chambers.

Their results suggest that DNA could keep its double-stranded structure at room temperature in a vacuum for as long as 35 years. "At the very low temperatures of space, the complexes would survive for a very long time—nearly indefinitely", says Williams. (Source: *New Scientist*, 10 October 1998)

Australian scientists in world-first cell discovery

A team of Australian scientists from CSIRO and the Biomolecular Research Institute has achieved a world-first advance by describing the structure of a vital receptor found on the surface of the body cells of all animals including humans. The discovery has major implications for understanding of the mechanisms behind growth and development, and diseases such as diabetes and many forms of cancer.

The breakthrough was made by a team led by Dr. Colin Ward of CSIRO Molecular Science and crystallographer Dr. Tom Garrett of the BRI in Melbourne. The work was funded in part by Biota Holdings Limited and the Federal Government's AusIndustry programme.

The team's goal is to understand the atomic structure of a particular family of receptor sites on the cell surface which detect chemical messengers such as insulin, IGF (or insulin-like growth factor) and EGF (epidermal growth factor).

Receptors are a vital link in the body's command chain. Messenger chemicals like hormones and growth factors attach and switch on their special receptor, which in turn commands the cell to perform particular tasks, such as to grow or to

process sugar. The team is the first to clarify the structure of half of the IGF receptor, marking a major scientific milestone in a field of research that has been running since the late 1960s when the 3D structure of insulin was determined.

"The IGF, insulin and EGF receptors are all in the same family and their structures are expected to be 90 per cent similar—so understanding the crystal structure of the IGF receptor helps us to understand most of the structure of all three", Dr. Ward explains. "IGF is important to the body's normal growth and development—but when it gets out of control it can also cause the growth of cancer cells. We hope this work in time will lead to a better understanding of ways to control certain cancers. This is a milestone in that process—but there is still a long, long way to go."

To understand the IGF receptor's structure, large quantities of the receptor fragment were produced in animal cells and purified to a very high level. The next step was to grow crystals from this material, much like salt crystals growing in a saline solution. The big difference is that the team's target crystal has over 7,000 atoms, whereas salt has just two.

The crystals were then bombarded with X-rays, yielding diffraction patterns. A powerful computer was then used to construct an image of the receptor from the diffraction data. From this Dr. Garrett was able to work out the location of each atom in the receptor and build a 3D structure for this protein molecule.

Three years ago a US team clarified the structure of the portion of the receptor that lies inside the cell. The Australians have now described the structure of half of the receptor that lies outside, on the cell surface. "The next step is to get the whole thing because the remaining parts of the receptor are also important for binding and biological action—and that should tell us a lot more about how these important chemicals communicate with the body. That, in turn, will help us to manipulate their effects and, hopefully, treat diseases like diabetes and cancer more effectively", said Dr. Ward. (Source: *Australasian Biotechnology*, Vol. 8, No. 4, August 1998)

Light trap

Chemists at the National Institute of Standards and Technology have succeeded in mimicking photosynthesis. After a decade of work the researchers have found that iron and cobalt containing catalysts (metalloprophyrins) similar to those in plants, can use the energy of light to convert carbon dioxide into other organic molecules. (Source: *Technology Ireland*, July/August 1998)

Fullerenes hotwired to DNA

A new approach for preparing molecular assemblies at the nanometer scale may one day allow the construction of microtransistors and miniaturized devices. By exploiting electrostatic interactions with the phosphate groups on DNA's backbone, University of South Carolina researchers, led by James Tour, have shown that it is possible to organize cationic derivatives of fullerenes—C_{60} aromatic carbon compounds shaped like soccer balls—into molecular assemblies using phiX174 DNA as a template. The complexes, about 1 micron long, can be obtained by a rapid single-step method and are easily imaged by electron microscopy without the need for heavy-metal staining. As well as trying to covalently link the fullerene units to obtain more rigid polyfullerenes (and thus dispense with the need for a DNA scaffold), the team is looking at semi-conducting properties of these complexes. (Source: *Nature Biotechnology*, Vol. 16, August 1998)

Synthetic hormone created

Researchers at Ligand Pharmaceuticals (San Diego, CA) and SmithKline Beecham (King of Prussia, PA) have discovered the first non-peptide small molecule capable of mimicking the action of a protein hormone *in vivo*, possibly opening the way to the development of compounds with greater pharmaceutical utility than biologically produced peptides. Using an assay for granulocyte colony-stimulating factor (G-CSF) receptor activation, the scientists identified the molecule from a panel of synthetic compounds produced at SmithKline. The highly symmetrical chemical is believed to act in a manner similar to G-CSF, which induces dimerization of its receptor, initiates proliferation of neutrophils, and is often administered to patients suffering from neutropenia. Unlike the hormone, though, the new compound is species-specific and appears to work only on mouse cells. "The mouse and human receptors are 66 per cent identical", explains Peter Lamb, Associate Director of Transcription Research at Ligand. Lamb adds that the result is an important proof of concept, as the two companies are also searching for small molecules that may act on the erythropoietin, thrombopoietin, and leptin receptors. (Source: *Nature Biotechnology*, Vol. 16, August 1998)

Multitalented enzyme yields secrets

Dehydroquinate synthase (DHQS), for many years an enzymological enigma, has yielded some of its secrets in a new study with implications for both antibiotic development and industrial biotechnology. Catalysing a key reaction in the shikimate pathway, through which many organisms synthesize aromatic compounds, DHQS appears to carry out several distinct reaction steps in a single active site. Researchers had hypothesized that many of the steps might occur spontaneously, with the enzyme present as a passive bystander. Scientists in the United Kingdom and United States report that the three-dimensional structure of DHQS overturns this model, showing that the enzyme could in fact play an active role in each reaction. While modifying and accelerating the multistep enzyme may eventually prove useful in industrial applications, more immediate goals include the rational design of compounds that inhibit it. Because DHQS is found in many fungi, plants, pathogenic bacteria and protozoans, but not in mammals, it is an attractive target for developing broad-spectrum antibiotics. (Source: *Nature Biotechnology*, Vol. 16, August 1998)

Benign skin tumours tied to elevated risk of cancer death

A usually non-fatal kind of skin cancer makes its victims more likely to die from other types of cancers, researchers say.

In a new study, published in the *Journal of the American Medical Association* (JAMA), scientists found that people with a history of non-melanoma skin cancer (NMSC) are at a 20-30 per cent greater risk of dying from other types of cancer than people without NMSC.

These types of cancers are usually treated and removed in a doctor's office, without any follow-up.

The study was designed to determine whether a history of non-melanoma skin cancer coincided with an increased risk of dying from other cancers.

The participants in the study were drawn from 508,353 men and 676,306 women who completed a 4-page questionnaire in 1982. Mortality rates were tracked through 31 December 1994.

The researchers concluded that men with a history of NMSC are 30 per cent more likely to die from cancer. Women who also reported incidences of skin cancer are 26 per cent more likely to die from cancer.

The study also compared mortality from specific types of cancer. For women with a history of NMSC, there was a 34 per cent greater risk of death from breast cancer than for patients without NMSC. Men with a history of NMSC had a greater risk of death from cancer of the salivary glands, prostate cancer, cancer of the testis, and urinary bladder cancer.

Both male and female patients were at greater risk of death from melanoma, cancer of the pharynx, lung cancer and non-Hodgkin's lymphoma.

The study suggested the link between getting skin cancer and dying from another type of cancer might be found in the patient's increased exposure to UV rays from the sun. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 21 September 1998)

E. APPLICATIONS

Pharmaceutical and medical applications

Rotavirus vaccine shows promise

Rotavirus is the most common cause of severe diarrhoeal disease in infants all over the world and an important public health problem, particularly in developing countries. The virus is believed to be responsible for 125 million cases of diarrhoea each year with 600,000 to 870,000 deaths. The incidence of severe and often fatal rotavirus diarrhoea is particularly high in developing countries. Several vaccine candidates have been evaluated in recent years and recent results obtained with a quadrivalent rhesus rotavirus vaccine (RRV-TV), the only vaccine licensed so far, has raised hopes that an effective vaccine will soon be available for widespread use.

Although trials in developed countries (USA and Finland) have shown this vaccine to be highly effective in preventing severe rotaviral diarrhoea, the ultimate test is its efficacy in a Third World setting. It is thus most encouraging that recent results of a vaccine trial in Venezuela, where 2,207 infants received three oral doses (4 x 10^5PFU/dose) of the RRV-TV vaccine, showed that it gave 88 per cent protection against severe rotaviral diarrhoea, 75 per cent protection against dehydration and produced a 70 per cent reduction in hospital admissions. The overall efficacy against a first episode of rotavirus diarrhoea was 48 per cent. It is now imperative to determine that the high level of protection seen against severe diarrhoea in Venezuela can be reproduced in other developing countries, especially in Asia.

The encouraging results of these vaccine trials seems particularly apt as 1998 marks the 25th anniversary of the discovery of rotavirus by Australian virologist Ruth Bishop who first described the agent in 1973. (Source: *Australasian Biotechnology*, Vol. 8, No. 3, June 1998.

TB or not TB

A new tuberculosis therapy that could help to eliminate virulent drug-resistant strains of the disease has won approval from the US Food and Drug Administration (FDA) despite being less potent than existing medicines.

The drug, rifapentine, won approval because it is simpler to use than existing drugs. The FDA believes this will lead to more patients completing their course of therapy, reducing the chance of resistant strains emerging.

TB patients currently have to take a combination of drugs every day for two months, then twice a week for a further four months. Many do not complete the rigorous course, allowing a few TB bacteria to survive in their lungs. These bacteria are often resistant to one or more of the drugs.

Rifapentine simplifies the second phase of the regime to a single weekly dose. Clinical trials indicate that 10 per cent of patients on rifapentine suffer a relapse—twice as many as those on the old drug rifampin—but the FDA believes that better compliance will balance this out.

Rifapentine, which will be marketed in the US as *Priftin* by Hoechst Marion Roussell, is the first new TB therapy on the market for 25 years. The company hopes to submit the drug for regulatory approval in Europe later.

TB kills more people worldwide than any other infectious disease. (Source: *Chemistry & Industry*, 6 July 1998)

Gene therapy shows promise

French researchers have developed an improved delivery method for one of the proteins recently touted as "a cure for cancer". The team injected mice with a virus carrying the gene for the protein angiostatin, halting tumour growth.

Angiostatin and endostatin fight cancer by blocking the growth of new blood vessels around a tumour. Deprived of its blood supply, the tumour shrinks and eventually dies.

Treatment with the proteins themselves requires constant delivery of the drugs, a process which is both costly and labour-intensive. The new method, developed by a team based at the Institut Gustave Roussy in Villejuif, France, uses a single injection of a harmless but infectious virus carrying the gene for angiostatin.

When injected into the tumour, the gene stimulates local secretion of angiostatin, blocking the blood supply to the cancer. A single injection inhibits the growth of rat and human tumours in mice by over 80 per cent. The team also found that

injecting the gene into non-cancerous tissues could help prevent cancer from developing.

The gene transfer method is more efficient for achieving a constant, long-lasting concentration of angiostatin at the tumour, the team says. It is also much cheaper than purifying large amounts of the protein from blood serum. The team hopes that an improved delivery method could completely halt tumour growth. (Source: *Chemistry & Industry*, 1 June 1998.

Gene therapy for haemophilia

Katherine High and her colleagues at the Children's Hospital of Philadelphia (Pennsylvania, PA) in collaboration with Avigen (Alameda, CA) have obtained extended and stable expression of the coagulation factor IX in a dog model of haemophilia B. Haemophilia B is one of the most common forms of haemophilia and results from a deficiency of normal factor IX. In research presented at the inaugural meeting of the American Society for Gene Therapy in Seattle, WA, High reported that a single intramuscular injection of an adenoassociated virus vector carrying the gene for canine factor IX resulted in stable expression of the gene for nine months. The gene therapy resulted in a dose-dependent increase in plasma levels of factor IX. The animals demonstrated improved clotting time and showed no toxic effects associated with the treatment. Avigen plans to extend the work to human trials by the end of 1998. (Source: *Nature Biotechnology*, Vol. 16, July 1998)

Cooking tumours to death

Cauterising tumours with radio waves may provide a less invasive alternative to surgery for liver cancer and many other types of tumour, say two cancer specialists.

Surgery to remove a tumour can prevent cancer spreading throughout the body. But in some cases, doctors decide not to operate, perhaps because the tumour is too close to an artery or because the patient is too weak to survive the operation. Sometimes chemotherapy can be used to kill the tumour cells. But these drugs kill healthy cells, too, and can make the patient feel very ill.

Radiologist John McGahan and oncologist Philip Schneider of the University of California at Davis are developing another alternative treatment, which is known as radio-frequency ablation. It involves heating the diseased cells with a fine electrode, and is similar to the traditional technique for cauterising blood vessels.

To treat liver cancers, the researchers insert a flexible catheter through a patient's abdomen, using an ultrasound scan to guide it into position against the tumour. They then run a cluster of eight to ten wires through the catheter. When the wires reach the end of the tube, they fan out and penetrate the tumour.

A very high-frequency current in the wires causes nearby atoms to vibrate, heating the tissue from within just as a microwave cooker heats food. It takes 10 minutes to kill all the cells in a region measuring 3 to 5 centimetres across. The technique can be used to destroy up to five liver tumours in a single session. Patients receiving this treatment can leave hospital much sooner than those who undergo conventional surgery.

However, radio-frequency ablation does have serious side effects. The liver contains many biochemically active substances that leak into the body from the dead cells. As a result, patients may become feverish or nauseous. And if the treatment damages nerves near the liver's surface, patients may feel chest or abdominal pain for up to a week.

The California team have so far tested their treatment on a dozen patients. Nine of the twelve have been free of cancer for more than a year. Left untreated, liver cancer is invariably fatal. Schneider expects that several more years of evaluation will be needed before the procedure becomes common.

McGahan says radio-frequency ablation could treat benign tumours as well as cancer. For example, he recently removed a painful bone tumour from a girl's leg by inserting the wire through a small hole in her femur. (Source: *New Scientist*, 10 October 1998)

Angiogenesis drugs boost power of cancer killing radiotherapy

Combining angiogenesis agents with radiation appears to fight cancer better than either therapy alone.

Although the research was conducted in mice, the study suggests that just a small dose of angiostatin could give a big boost to radiotherapy, said Ralph Weichselbaum, the chairman of radiation oncology at the University of Chicago. The strategy also appears to leave normal tissues unharmed, and may avoid some of the side effects of cancer therapy, he said.

In the study, Weichselbaum and collaborators from Northwestern University tested the approach in mice, with tumours of human or mouse origin. Together, angiostatin and radiation resulted in a 64 per cent drop in tumour size, the scientists found.

Weichselbaum has also conducted similar studies with endostatin and he said the results, which are not yet published, are "awesome".

Weichselbaum believes this combination treatment is "clinically feasible" for use against solid tumours, and could be especially effective in brain cancer, and head and neck tumours, in which tumour size is significant and dangerous, and in metastatic disease. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 20 July 1998)

DNA vaccine protects monkeys from rabies; may help Third World

A new kind of vaccine could reduce the worldwide death toll from rabies, which claims 40,000 lives a year, mostly in developing countries.

The vaccine, composed of a rabies gene packaged in a DNA carrier, protected eight out of eight experimental monkeys from lethal doses of the virus, said researchers from the Rocky Mountain Laboratories in Hamilton, Mt., part of the National Institute of Allergy and Infectious Diseases.

Virologist Donald L. Lodmell said, "There is no gray area. It was a 100 per cent survival".

The DNA vaccine also appeared to produce an immune response against the "global spectrum of rabies virus", something the scientists confirmed through test-tube experiments, he said.

Mass immunization with the DNA vaccine, which costs just pennies to produce, may be the answer to the rampant rabies problems in developing countries. The DNA vaccine could be useful in these regions because it is inexpensive and easy to administer.

DNA vaccines would be a welcome alternative to the methods used to produce rabies vaccines in poor countries, in which the vaccine is extracted from the brains of infected animals, a technique that comes along with serious, even deadly, side effects.

Lodmell led the research, in which eight monkeys were given the DNA vaccine through either injection or the gene gun, a method in which helium is used to propel DNA-coated gold beads into the skin.

Two other monkeys were given current commercially available vaccine, which is composed of an inactivated rabies virus. Two control monkeys received no vaccine.

After booster at about six months, the animals were given "a huge dose" of virus, hundreds of times higher than the amount that would be transmitted by an infected animal, said Lodmell.

All of the animals given vaccines remained disease-free, while the two unvaccinated monkeys quickly developed symptoms of rabies, said Lodmell.

The DNA vaccines, which are easy to make and transport because they require no refrigeration, could be useful in areas where the virus is endemic and people are too poor to afford the current vaccines.

One major drawback is that it takes a long time—up to a month—for these preparations to provoke an immune response, and that rules out their potential as an alternative to the current post-exposure vaccines.

Lodmell is aware of this drawback, and agrees that DNA vaccines are not ready for post-exposure use. Rabies symptoms can appear in as short a time as a week and, after that, nothing can be done to help.

His group is working on making the immune response faster and stronger, by tinkering with the formulation, but he believes that the vaccine in the form it is in right now could prevent thousands of people from becoming sick in the Third World.

However, he said, that it will take involvement by the World Health Organization or the governments of the countries hardest hit by rabies. As a basic researcher, he said, he cannot move the vaccine forward to the human experiments needed to show it works, and vaccine companies would most likely have little interest in developing it, because the potential for profit is so low. (Source: *McGraw Hill's Biotechnology Newswatch,* 17 August 1998)

Breast cancer breakthrough

The world's first preventative treatment for cancer could be on the way with news that large-scale trials with tamoxifen have shown that it can reduce the incidence of breast cancer by 45 per cent. Tamoxifen, used for more than two decades to treat breast cancer, is produced by Zeneca and marketed as *Nolvadex.*

The US study of more than 13,000 women, half of them taking tamoxifen and half of them taking placebo, was stopped a year early by the US National Cancer Institute (NCI) because of the dramatic findings. The NCI decided that tamoxifen should be offered to all the women taking part in the study.

"This is the first time in history that we have evidence that breast cancer cannot only be treated but also be prevented", said Bernard Fisher, scientific director of the study. The drug works by countering the effects of oestrogen, which stimulates breast tumour growth. It was originally developed by ICI in the 1960s and first launched in the UK in 1973.

An unexpected bonus of taking tamoxifen is that there is evidence that it is also effective against osteoporosis, although there are some side effects. Side effects linked to the drug include hot flushes, irregular periods and weight gain. The drug can also increase a woman's chances of cancer of the uterus, a blood clot in the lung or pulmonary embolism, and deep vein thrombosis.

Nolvadex is the best-selling drug for the treatment of breast cancer in the world. It had sales of more than $500 million last year. Although it is off-patent in all major markets outside the US it has retained its market share. It was the fourth-biggest selling cancer drug in the US in 1996, where it retains patent protection until 2002. (Source: *European Chemical News,* 13-19 April 1998)

Positive news on cancer drugs

Expectations have been raised about a leap forward in cancer treatment with positive news on a number of cancer drugs.

The share price of US biotechnology company EntreMed, based in Rockville, MD, trebled after news of its success in treating tumours in mice with the drugs angiostatin and endostatin. "The data are very impressive and compelling but it is still mouse data. We need clinical data in humans before we can anoint them as miracle drugs", said Jim Pluda, a cancer researcher at the National Cancer Institute (NCI) which is overseeing research in this area. The drugs are in pre-clinical development and it could be a year before they are tested in humans.

"There have been a number of compounds in the past that have cured mice and did not translate into efficacy in human clinical", added Pluda.

EntreMed already has a collaboration agreement with Bristol-Myers Squibb (BMS) on angiostatin and BMS is expected to enjoy first rights of negotiation for development of endostatin.

Roche has received accelerated approval in the US for its new anti-cancer drug *Xeloda* (capecitabine), for breast cancer. In a Phase II trial involving 162 patients, 18.5 per cent of patients experienced a reduction in tumour size of more than 50 per cent and a few experienced complete remission. (Source: *European Chemical News,* 11-17 May 1998)

Brain tumour drug blocks gene thought to be driving force of cancer

A new age in the treatment of brain cancer may be only a couple of years away with the launch of a Phase III trial for an agent that blocks the growth factor responsible for the tumour before it can signal a cell to begin growing wildly.

SU101, a small molecule inhibitor that blocks the transmission of the signals of the platelet-derived growth factor receptor (PDGF), is being tested against Procarbazine, the most common drug for patients with out-of-control brain cancer. If all goes well it could become the first signal transduction inhibitor to get an FDA approval for treatment of cancer.

PDGF is believed to be the driving oncogene in several types of brain, prostate, ovarian and non-small cell lung tumours.

In early trials there was a problem with swelling of the brain, but the dose has now been worked out so that the one significant side effect is under control.

Dr. Eric Wong, co-director of the Brain Tumor Center at Beth Israel Deaconess Medical Center, Boston, said that "other than the swelling it is pretty well tolerated". "Unlike other drugs, it can penetrate the blood-brain barrier and does not lower a patient's blood count, making it even less toxic".

In the Phase II trials SU101 kept tumours at bay for four months in 6 of the 12 patients who completed the regimen. The tumour size was reduced by at least 25 per cent in three of the six patients. To date 200 patients, all in terminal stages of the disease, have taken part in various SU101 trials. Nearly half showed tumour shrinkage or stable tumour size.

Wong is optimistic and thinks that an FDA approval for the drug could come as soon as 2001. He said he feels the drug can become the standard treatment for the tumour, even if it works at the same level as current therapy, due to the lack of side effects. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 20 July 1998)

TNT therapy to aim at brain tumours in Phase II trial

Techniclone plans to submit its Phase II protocol to the FDA for the treatment of glioma in the near future, for its Tumour Necrosis Therapy, or TNT.

The company plans to begin enrolling patients for the trial by the end of 1998, which will be held at the Medical University of South Carolina.

In addition, Techniclone recently received a grant from a major pharmaceutical company to conduct a Phase I/II trial of its TNT radiolabelled I131-chTNT-1B isotope in a variety of solid tumour types, including prostate, pancreatic, and liver cancer, in Mexico City.

This trial will also explore alternative delivery procedures, including intratumoural and intravenous injection of the drug.

The company also said it will release interim results from its Phase I trial in glioma shortly.

TNT is a universal delivery system for both therapeutic and diagnostic uses which is designed to avoid some of the most significant pitfalls seen to date with conventional monoclonal antibody therapy.

Specifically, TNT avoids the limitations of cell-surface antigens as monoclonal antibody (MAB) targets, as well as those caued by the poor blood supply in solid tumours; it enables the delivery of significantly higher doses of traditional chemotherapy and radiation to tumours than conventional monoclonal antibodies, with much less toxicity and fewer side effects, as it is accessible only to "leaky" necrotic tumour cells.

As the antibody and radiolabelled isotope is taken up by these cells, the radiation also spread to the surrounding cells, killing them, and the process continues in this way, killing the tumour from the inside out.

Whereas conventional monoclonal antibodies target the antibody to antigens on the surface of the cancer cell membrane, such cell-surface antigens are unstable and modulate, causing the antigen target often to disappear.

Also, the same antigen is often expressed on normal, healthy cells, causing high toxicity and adverse side effects, and lastly, cell-surface antigens vary greatly among tumours, requiring a different MAB to be developed for each type of cancer.

The TNT delivery system targets antigens on the DNA within the nucleus.

In contrast to normal tissue, in which the outer cell membrane is healthy and non-leaky to large molecules like MABs, cancer cells undergo rapid degeneration, which results in areas of necrosis, in which the cell membrane is leaky, and porous.

By using the DNA within the cancer cell's nucleus as its target, the therapy reliably attaches to the DNA "anchor", which does not modulate or disappear. So healthy membranes are not permeated by the therapy, and the DNA anchor is universal to all types of cancer cells.

Solid tumours have inadequate blood supply in the interior, which hastens the process of necrosis but is not accessible by conventional MAB therapies. (Source: *McGraw Hill's Biotechnology Newswatch*, 7 September 1998)

Delivery of DNA vaccines

Glaxo Wellcome with PowderJect Pharmaceuticals will develop and commercialize PowderJect's technology to deliver a range of DNA vaccines.

The PowderJect delivery system injects microscopic particles (1-3μm in diameter) through the skin, after accelerating them to supersonic speeds with a helium gas stream. The particles are made of gold coated with DNA vaccine. Once inside the body, the DNA elements separate from the inert carrier particles and provoke an immune response.

The collaboration initially covers the development and worldwide use of the PowderJect system to deliver a hepatitis B DNA vaccine which PowderJect has in Phase I clinical trials in the US. An option is included to extend the collaboration to a further ten DNA products. These include an HIV therapeutic vaccine, two therapeutic DNA vaccines for undisclosed infectious diseases, cancer DNA vaccines using two undisclosed antigens, and four additional DNA vaccines to be selected at a later date. However, there are no definite dates for Phase II and III trials with the hepatitis vaccine, and it could be the mid-2000s before the products are commercially available.

PowderJect is also developing its delivery technology for other DNA vaccines—for autoimmune diseases, allergies and other immunopathologies—and other types of drugs, such as local anaesthetics with Chiroscience, and therapeutic proteins with Boehringer Mannheim. (Source: *European Chemical News*, 9-15 March 1998)

Cancer researchers strike gold

Compounds made from gold and silver may make good anticancer drugs, say researchers in Australia and New Zealand.

The unpleasant side effects of chemotherapy often come from the drugs' inability to selectively attack cancer cells. Recent research suggests that drugs may be able to kill cancer cells by targeting their energy-producing mitochondria, which have more highly charged membranes than those in normal cells.

To develop positively charged drugs that could cross the mitochondrial membrane, Susan Berners-Price and her team at Griffith University in Brisbane, Australia, with Mark McKeage of the University of Auckland in New Zealand, turned to gold and silver.

They found that in mice, these compounds could kill ovarian cancer cells that were resistant to a common cancer drug called cisplatin that has no charge and targets DNA.

The researchers speculate that the positive charge on the new molecules helps them to penetrate the mitochondria and thus disrupt the cells' source of energy.

By slightly altering an organic part of the molecule, Berners-Price says she can change both the selectivity of the compounds and their potency against cancer cells. (Source: *New Scientist*, 12 September 1998)

Progress in development of edible vaccines

The use of transgenic plants expressing antigens from pathogenic microbes has led to the idea of "edible" vaccines. Earlier experimental studies with pathogenic *E. coli* and also with *V. cholerae* have shown that mice fed on transgenic potatoes expressing toxin genes produced a significant immune response and were protected from challenge with the virulent toxin. In the next significant step, researchers have

recently demonstrated the "proof-of-principle" in preliminary human trials of edible vaccines. Carol Tackett and colleagues (Center for Vaccine Development, Baltimore, MD, USA) in collaboration with Charles Arntzen (Boyce Thompson Institute, Ithaca, NY, USA) showed that 90 per cent of human volunteers who had ingested small amounts (100 g) of raw potatoes expressing the LAB toxin gene of *E. coli* developed striking serum I.G. anti-LT responses. Most of these volunteers also exhibited a mucosal secretory I.a. response. Although many practical obstacles have to be overcome before edible vaccines become a reality, the results of the first clinical trial are most encouraging. (Source: *Australasian Biotechnology*, Vol. 8, No. 3, June 1998)

Potatoes may prevent diabetes

Scientists from California's Lama Linda University, have shown that potatoes carrying insulin DNA prevented a form of diabetes—known as Type I or juvenile diabetes—in mice.

Lead researcher William H. R. Langridge said this experiment opens the door to using genetically engineered potatoes—so-called edible vaccines—to fight a range of autoimmune diseases, such as multiple sclerosis and arthritis. The same approach may also be developed to combat the difficult problem of preventing rejection of transplanted organs, he says.

The insulin-bearing potatoes acted like a vaccine against Type I or juvenile diabetes, by somehow training the immune system to tolerate the protein, shutting down the inappropriate immune response.

Langridge said that potatoes carrying the insulin gene alone were ineffective. But it worked well when the scientists hooked the insulin gene to the cholera toxin DNA.

The study is published in October's issue of the journal *Nature Biotechnology*. (Source: *McGraw Hill's Biotechnology Newswatch*, 5 October 1998)

Human antibodies produced in field crops enter trials

Trials are to begin for a strain of corn genetically engineered by Agracetus of Middleton, WI, to secrete human antibodies, according to an article in *Scientific American*. The article says that a pharmaceutical partner of Agracetus plans to begin injecting cancer patients with doses of up to 250 mg of antibodies purified from corn seeds.

The story says that to date, biotechnology-based techniques to bring antibody-based drugs to market have failed because they are developed from animal rather than human cells, The costs involved are also prohibitive. Now, says Vikram M. Paradkar of Agracetus, "plantibodies" could solve the problem. By transplanting a human gene into corn reproductive cells and adding other DNA that boosts production of the foreign protein, Agracetus has created a strain that yields about 1.5 kg of pharmaceutical-quality antibodies per acre of corn. Paradkar says that just 30 acres of the transgenic corn would be needed to fill the US market.

Plantibodies might reduce another risk as well: human diseases cannot grow in plants. So although Agracetus must ensure that its plantibodies are free from pesticides and other kinds of contaminants, it can forego the expensive screening for viruses and bacterial toxins required for fermentation technology. (Source: *The AgBiotech Bulletin*, January 1998)

Group mass produces Taxol

Scientists from Okayama University of Science and Belgium Free University have developed a method for mass production of 10-deacetyl-baccatin, a paclitaxel intermediate.

Paclitaxel is marketed as Taxol by Bristol-Myers Squibb.

Disinfected leaves of the Japanese yew tree were cultivated for the process, and cultivation was stimulated by plant hormones.

After two to four weeks, cell colonies were found on the leaves that contained 115 mg of 10-deacetyl-baccatin.

This method is 10 times as productive as existing processes. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 February 1998)

Vaccine raises antibodies against E. coli 0157

A genetically engineered vaccine given to a small group of courageous volunteers provoked an immune response to the often deadly *E. coli* 0157, raising the prospect of universal protection from the microbe.

The experimental vaccine was made by hooking a sugar chain from the surface of the bacteria to a genetically weakened version of the toxin produced by another bacterium, *Pseudomonas aeruginosa.*

The weakened toxin alerts the immune system to the sugar so that when it comes into the body on *E. coli* 0157 a full attack is mounted before the deadly strain can overwhelm a person's natural defences.

A dozen people died in Japan during a 1995 outbreak that sickened more than 10,000 and last year 20 elderly people died in Scotland in an outbreak that struck a senior citizens residence.

The *E. coli* strain is thought to be a result of a kind of natural genetic engineering. It is theorized that it swapped genes with the Shigella bacteria sometime during the 1970s, turning the usually benign resident of the human gut into a killer.

One of the problems with *E. coli* 0157 is that antibiotics are not effective and can often make the infection worse. Symptoms range from mild stomach upset and diarrhoea, to kidney failure and death. The very young, old and immune impaired are especially vulnerable.

The vaccine was created by scientists from the National Institute of Child Health and Human Development.

The agency's director, Dr. Duane Alexander, said that the volunteers showed protective antibody levels in their blood within a week of getting the vaccine, indicating that the vaccine could be used to stop a future epidemic in its tracks.

The volunteers were never exposed to *E. coli* 0157 during the 26-week study, so researchers do not know if they were actually protected from it. At the end of the study 97 per cent of the volunteers had antibody levels 10 times their pretest level.

The volunteers showed no serious side effects from the vaccine other than a slight irritation at the injection site. An unnamed firm in the Midwest is now testing it on cattle. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 February 1998)

A company's gift of AIDS drugs

Some African AIDS patients will soon have access to "triple therapy", the life-prolonging combination of anti-HIV drugs that has so far largely been the preserve of clinics in developed countries.

Hoffmann-La Roche, the Swiss-based drugs giant, says it will donate its drugs saquinavir and ddC, plus supplies of AZT purchased from Glaxo Wellcome, free to hospitals in Kenya, Tanzania, Uganda, Zimbabwe, Zambia, Malawi, Côte d'Ivoire and Cameroon. When the donation—worth more than £400,000—runs out, the drugs will be available at a discount. The firm is negotiating with the countries over a price they can afford. (Extracted from *New Scientist*, 26 September 1998)

Blood booster may block HIV entry

A drug used to boost white blood cells may prevent the virus that causes AIDS from entering human macrophages, according to Immunex.

Immunex researchers say that *in vitro* studies showed that Leukine inhibited the expression of the co-receptors CCR5 and CXCR4, which are needed by the AIDS virus to get into macrophages. They also found that Leukine-treated macrophages had up to a 100-fold decrease in the entry of HIV-1. The drug also increased the production of chemokines, molecules that also inhibit HIV entry.

In a Phase I clinical trial, 10 out of 20 HIV patients were given Leukine for eight weeks. Eight showed a 30 per cent rise in CD4 T cells. Only three on placebo had that kind of reaction. Viral loads also decreased in patients getting Leukine, something that happened in none of the placebo patients.

The AIDS Clinical Trial Group is currently conducting a 24-patient, multi-centre, pilot study to determine if Leukine can eliminate HIV from certain immune cells. Immunex is also conducting a 300-patient Phase III trial to evaluate Leukine in the prevention of infections and death in AIDS patients. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 February 1998)

Brighter future

A short course of the anti-AIDS drug AZT in the final month of pregnancy halves a mother's risk of passing HIV to her child. The results of a trial of the treatment in Thailand suggest that this is an effective and affordable way to reduce the number of babies born with HIV in developing countries.

"With 1600 babies born every day with HIV, most of them in the developing world, we need this treatment as soon as possible", says Joseph Saba, head of the UNAIDS Working Group on the Prevention of Mother to Child HIV.

Four years ago, research showed that a three-month course of AZT cut the risk of passing HIV to the baby by almost 70 per cent—from 1 in 6 to 1 in 20. This treatment is now standard in wealthy Western nations. However, the treatment was thought too complex and, at $800 a patient, expensive for most developing countries. The trial in Thailand aimed to check whether a shorter course of the drug would be effective.

The study of 393 pregnant women, funded by the US Centers for Disease Control and Prevention, found that women who took AZT during the last four weeks of pregnancy and during labour were 51 per cent less likely to transmit HIV to their child than those receiving dummy pills. The course costs a tenth of the standard treatment.

As soon as the results of the study were announced, the CDC and the US National Institutes of Health ordered an immediate end to the placebo arm of the trial, which had attracted strong criticism. Sabo argues that it was necessary to prove how effective the shorter treatment was.

UNAIDS and the French national AIDS research agency, ANRS, also told investigators in the trials they sponsor to halt placebo treatment. "Using placebo controls after the Thailand results cannot be justified", says Saba.

Representatives from CDC, NIH, and UNAIDS, from developing and Western countries will discuss how to introduce the treatment to as many countries as quickly as possible. (Source: *New Scientist*, 28 February 1998)

Possible Parkinson's therapy

Cells from the necks of patients with Parkinson's disease could be used to treat their symptoms, a study in Spain suggests.

The disease's tremors and rigidity are caused by the destruction of neurons that produce the brain chemical dopamine. A team led by José López-Barneo at Seville University relieved Parkinson-type symptoms in rats by transplanting certain neck cells into their brains. These cells release dopamine when the blood is low in oxygen.

The cells responded in the same way in the oxygen-poor environment of the brain. If this works in humans, transplant therapy could become much more common. Doctors now rely on dopamine cells from aborted foetuses. (Source: *New Scientist*, 28 February 1998)

Blood-brain barrier overcome

Until now there has been no effective way of using anticancer drugs to treat brain tumours. The reason for this is the blood-brain barrier which protects the brain from harmful toxins and germs. This membrane also blocks anticancer drugs. Working in collaboration with Russian colleagues, scientists at Frankfurt University have now found a way of transferring the drug doxorubicin into the brains of rats. The researchers linked the anticancer drug to minute spheres of biodegradable plastic, covered it with process agents, and injected it into the rats. If the trials can be successfully repeated on humans, the procedure could open up new therapy techniques for tumours, Parkinson's disease, and Alzheimer's. (Source: *Deutschland No. 3*, June 1998)

Early intervention is the key in new treatment for blood diseases

By injecting foetuses with primordial red blood cells, doctors hope to revolutionize the treatment of sickle cell disease and thalassaemia. The procedure has been tried before with mixed results. But Rhodri Jones and his colleagues at the Queen's Medical Centre in Nottingham believe they are now armed with crucial information that will increase their chances of success.

Bone marrow transplants can provide a source of healthy blood cells, but fewer than 1 per cent of patients are lucky enough to find a donor whose bone marrow matches their tissue type. A mismatch means the transplant is rejected. No tissue matching is needed in the new procedure, so Jones and his colleagues hope they will be able to treat many more patients.

Jones and his colleagues hope to give the foetuses a perpetual source of normal red blood cells by injecting them with cells from healthy donors. The cells, called haematopoietic stem cells, mature to produce red blood cells and more copies of themselves. The researchers hope that the donated cells and their descendants will eventually dominate the blood supply and displace the diseased cells.

DNA analysis can pick up both sickle cell disease and thalassaemia by 12 weeks into pregnancy. If a problem is detected, just 1 millilitre of donated stem cells is injected with a fine syringe through the mother's abdomen and into the abdomen of the foetus. Ultrasound scans let the doctors inject the cells into the right place.

Once inside the body, the transplanted cells migrate to the liver, where they mature into red blood cells, says Jones.

Jones says that the procedure has already been attempted around 25 times by other teams in Sweden, France, Italy and the US, but some of the operations have failed because in some cases the doctors did not appreciate that the transplant must be carried out before the foetus reaches 14 weeks.

Before this time, the foetus has not developed its immune system, so it accepts the transplanted stem cells as "self", even if there is not a tissue match. And after 14 weeks, cells no longer migrate to the liver. Instead they accumulate in the bone marrow or the spleen, where they do not contribute to normal red cell production in the developing foetus.

Jones and his colleagues have built a special bank of stem cells in Nottingham. The cells are extracted from donated adult bone marrow and from discarded umbilical cords. The researchers remove all the white blood cells that might attack the foetus. (Source: *New Scientist*, 13 December 1997)

Neutrons tame tumours

An attempt to use neutrons to kill glioblastoma brain tumours is showing promise.

Patients swallow a boron compound, which is taken up preferentially by cancer cells. They are then irradiated with a neutron beam at the European Commission's High Flux Reactor at Petten in the Netherlands. The neutrons make the boron emit alpha particles, which kill cancer cells.

The tumours in two patients appear to have stopped growing.

The trials were supposed to start in 1991 but it has taken until now to obtain official approval from all the countries supplying patients. (Source: *New Scientist*, 13 December 1997)

Compounds for use in cancer treatment

BioResearch Ireland (BRI), has discovered that a specific Monoamine oxidase (MAO) inhibitor, code-named GPX-325, can protect normal healthy cell lines and tissue explants against the degenerative effects of irradiation while affording no protection to tumorigenic cells or explants. It has also been discovered that normal cells were selectively protected against the cytotoxic effects of three different classes of chemotherapeutic agents by the presence of GPX-325. BRI has filed a PCT Patent Application to cover the use of MAO inhibitors as Cytoprotective Agents, and has signed an Evaluation and a Licence Option Agreement with the US Company, Gem Pharmaceuticals, Inc.

The ability of MAO inhibitors to protect/rescue normal cells from the toxic side effects of irradiation or the administration of chemotherapeutic agents may have very positive implications for the treatment of cancer. In conjunction with the administration of GPX-325, higher, and potentially more cytotoxic, doses of radiation or chemotherapeutics may be administered to tumorigenic tissue while the surrounding normal cells remain relatively undamaged.

BioResearch Ireland is a research organization established by the Irish Government in 1988, as a partnership between the universities and the Government. The primary objective of BRI is to commercialize biotechnology opportunities arising from research in Ireland's university sector, with the ultimate goal of developing the biotechnology infrastructure in Ireland, providing a basis for the establishment of new biotechnology companies.

Further details: Séamus O'Hara, Business Development Manager, BioResearch Ireland, Forbairt, Glasnevin, Dublin 9, Ireland. Tel.: +353-1-8370177; Fax: +353-1-8370176; e-mail: oharas@biores-irl.ie (Source: *News Release*, 10 June 1998)

Could a household detergent wash cancer away?

A detergent in household cleaners may be a powerful weapon against multidrug-resistant tumours. Canadian researchers say it greatly enhances the effect of cancer-killing drugs by clogging a protein that cancer cells use to pump out the drugs.

For over 20 years, researchers have known that if cancer cells produce the P-glycoprotein pump, they become resistant to a wide range of drugs. But the same protein is also produced in some healthy tissues, such as the gastrointestinal tract and the kidney.

To find out, Jeffrey Charuk, a biochemist at the University of Toronto focused on the kidney, since whatever the renal P-glycoprotein binds to would be pumped into the urine. The problem was that the pump binds to many commonly used drugs, so the researchers needed a source of urine guaranteed to be drug-free. Charuk volunteered and collected samples of his urine for analysis for three years.

The researchers then treated multidrug-resistant cancer cells with the urine. They reasoned that any substance that binds to P-glycoprotein should enhance the ability of anti-cancer drugs to destroy tumour cells, since the protein would pump out this substance rather than the drugs. Sure enough, they were able to purify a chemical that enhanced the killing ability of chemotherapy drugs a hundredfold. Even by itself, this substance had some cancer-killing ability.

The surprise was the substance's identity: nonylphenol ethoxylate (NPE), a synthetic detergent used in dishwasher powder, window cleaners and hard surface cleansers.

The work suggests that P-glycoprotein evolved to eliminate natural toxins in plants, such as taxol. The irony is that some of these toxins are now being used as anticancer agents.

Charuk believes detergents like NPE are attractive candidates for new anticancer agents because they are inexpensive and abundant—more than 600,000 tonnes are produced each year. They are also less toxic than most anti-cancer drugs and are easily flushed out of the body. Charuk's group is now eager to test the effect of NPE on tumours in laboratory animals. (Source: *New Scientist*, 6 June 1998)

Colds frozen out

A cure for the common cold may be within reach after all: In a few months, researchers in California will test a drug that stops the cold virus replicating.

Previous attempts focused on keeping the virus out of the cells by blocking the interaction between the virus and cell surfaces, but there are more than 100 different varieties of the human rhinovirus with widely varying surface proteins, says Amy Patick, a virologist at Agouron Pharmaceuticals in La Jolla, so one drug could not work against them all.

Instead, her team decided to target 3C protease, the enzyme that lets the virus replicate itself inside cells. Protease inhibitors are best known for the way they revolutionized

treatment of HIV by disabling its protease enzyme. Patick's team designed a compound that binds to 3C protease of the rhinovirus and disables it.

Adding the compound to infected cells stopped them dying. A small amount of the compound saved 90 per cent of the cells—and it worked against all 46 different variants of the virus that the team tested.

Clinical trials of the drug, which will be taken as a nasal spray, should start within the next few months. While anyone with a cold could take the drug, the company plans to market it to people with cystic fibrosis and other such illnesses that make them particularly vulnerable to rhinoviruses. (Source: *New Scientist*, 3 October 1998)

A mutant viral protein may soon go on trial as an asthma vaccine

A vaccine against asthma in children may be a step nearer, thanks to an insight into a virus linked to the disease. Scientists say a vaccine could be ready for trials within three years.

The key development concerns the so-called respiratory syncytial virus (RSV), which can cause lung inflammation. Air pollutants and common allergens such as house dust mites are thought to make symptoms worse in people with asthma, but many experts believe infection by RSV may well be the underlying cause of asthma in roughly a third of all sufferers.

Peter Openshaw of Imperial College School of Medicine in London and his colleagues report that they have identified the active part of a substance the virus produces, called the G protein. Earlier work suggested the G protein triggers a huge influx of inflammatory cells into the lungs of infected animals. This influx throws the immune system off balance, switching it into the mode used for fighting infections of parasitic worms. Clogging mucous is produced, which may help the virus spread to other people by making victims cough and sneeze.

By testing a range of mutant G proteins, Openshaw and his colleagues showed that only a small sequence of the protein was necessary to disrupt the immune system in mice. When the G protein lacked this sequence, the researchers could protect mice against infection—without triggering inflammation.

Colleagues of Openshaw at the National Institutes of Health near Washington, D.C. hope to test live-modified vaccines with altered G proteins, to boost children's immunity to RSV, within three years.

However, the researchers warn that there are still safety concerns. A trial of an earlier RSV vaccine in the 1960s floundered when immunized children turned out to be many times more likely to need hospital treatment than controls when later exposed to RSV. (Source: *New Scientist*, 13 June 1998)

New insulin delivery technique

Researchers have found that acoustic waves can make the skin temporarily permeable, so doctors can sample body fluids or inject medication without piercing the skin. They can generate these acoustic waves using ultrasound or a shockwave from a pulsed laser.

Clinical trials are already under way on an ultrasonic system for measuring glucose levels in diabetics, developed at the Massachusetts Institute of Technology in Cambridge, MA. Massachusetts General Hospital in Boston is also testing a drug-delivery system that uses laser technology.

Sound waves temporarily change the structure of the impermeable outer layer of skin, which consists of dead skin cells encased in layers of fat molecules and is between 10 and 15 micrometres thick. A few minutes of low-frequency ultrasound—sound greater in frequency than 20 kilohertz, at the threshold of human hearing—creates tiny cavities.

These holes allow large molecules to pass through the fat layer for several hours, says Joseph Kost, a professor of chemical engineering from Ben Gurion University of the Negev in Beersheeva, Israel, who is at MIT.

With MIT's Bob Langer and Samir Mitragotri, Kost is focusing initially on glucose testing. The non-invasive acoustic technique makes testing much easier—all patients have to do is place a glucose-monitoring patch over the permeable spot.

Initial tests used a desktop device, but Kost and Langer have formed a company to develop a hand-held, battery-powered ultrasound generator for use at home. They plan clinical tests of a similar system to deliver insulin or other drugs.

Meanwhile, Apostolos Doukas at Massachusetts General is using laser pulses to generate the acoustic waves that make the skin permeable. He fires a short laser pulse at a plastic disc laid on the skin, producing a stress wave that lasts 0.2 microseconds to create the temporary pores. Drugs applied between the disc and the skin can then diffuse through the pores while the barrier remains open.

As with the ultrasonic technique, patients in the trials said they felt no pain.

Small pulsed lasers can also penetrate the skin, but they drill holes which, like needle pricks, can cause pain and take time to heal. (Source: *New Scientist*, 20 June 1998)

Foreign lessons

Inserting a foreign gene into a patient's bone-marrow cells could help their immune system to accept transplants from non-human donors.

Although drugs can stop the body from rejecting a human transplant, the immune system will not accept animal organs for very long. This is because the single most common antibody in human blood is directed against the carbohydrate αGal, found in the tissues of all non-primate mammals.

John Iacomini at the Harvard Medical School in Boston and his colleagues have found a way to persuade antibodies not to attack cells that are making αGal. To try the technique in lab animals, they used mice that were genetically engineered so that, like humans, they lacked the gene that allows αGal to be made and instead produced αGal antibodies.

Then the researchers extracted antibody-producing B-cells from bone marrow, added the αGal gene, and transplanted them back in. These cells produced αGal, and the rest of the body saw it as "self". Antibodies in the mouse serum did not attack pig kidney cells in a dish.

Iacomini plans to use engineered marrow to try something like transplanting a pig's organ into a baboon. Other experts in the field are cautiously optimistic. (Source: *New Scientist*, 26 September 1998)

FDA approves treatment for Crohn's

Federal regulators have approved the first treatment specifically for Crohn's disease, an incurable bowel condition that causes diarrhoea, pain and, in severe cases, channels that bore through from intestines to the surface of the patient's skin to cause oozing wounds.

The drug is called infliximab and it will be marketed by biotechnology company Centocor, Inc., Malvern, PA., under the brand name Remicade.

Remicade is a genetically engineered antibody that blocks tumour necrosis factor alpha, which promotes inflammation.

Centocor said the drug is expected to be available nationally in late September or early October. It will cost about $450 per vial, and the average patient is expected to be given a three-vial treatment.

A clinical study of 108 patients showed that Remicade reduced symptoms of the disease in 82 per cent of those who did not have an adequate response to other therapies, compared to about 16 per cent of patients who were given a placebo, said Centocor spokesman Christopher Allman.

About half had a clinical remission, he said.

There are about 800,000 patients worldwide who have Crohn's and the company estimates about 140,000 patients in the US and 160,000 patients in Europe are candidates for the drug, he said.

In another study, the drug also reduced the number of fistulas. In the study of 94 patients, about 68 per cent of patients had half of their fistulas close up after treatment with Remicade, compared to 26 per cent of patients given a placebo.

More than half of the patients had all of their fistulas close up after treatment with the drug, said Allman. But in six patients, doctors found abscesses after a few months, a sign that the fistulas may be opening again.

Allman said Centocor is working with the FDA to design a clinical trial to examine the long-term and chronic use of the drug. The company is also investigating the use of the drug in rheumatoid arthritis and asthma. (Source: *McGraw Hill's Biotechnology Newswatch*, 7 September 1998)

Sugoid may work against hepatitis B

A spoonful of "sugoids" may help in the battle against hepatitis B, researchers in Philadelphia and Oxford say.

"Sugoids" are molecules that chemically resemble ordinary table sugar, but some of them have anti-viral activity, according to biochemist Timothy Block of Thomas Jefferson University in Philadelphia.

And one in particular—a sugoid whose chemical name is N-nonyl-DNJ—appears to block the reproduction of the hepatitis B virus in animal models.

Worldwide, more than 350 million people are infected with hepatitis B, and about 140 million of them will die, Block said.

Currently, there is a vaccine for the virus, but no completely effective treatment. Alpha-interferon is approved for the disease, but works in only about a third of patients, while lamivudine, a reverse transcriptase inhibitor, appears to work well but has yet to be approved. There are also fears that it may induce drug resistance.

Block, working with Novel Laureate Baruch Blumberg of the Fox Chase Cancer Center in Philadelphia and Raymond Dwek of Oxford University, has shown that N-nonyl-DNJ inhibits alpha-glucosidases in the cellular endoplasmic reticulum (ER).

The hepatitis B virus begins its self-assembly in the ER, Block said, and if the proteins involved are not glycosylated, the assembly bogs down, probably because folding and transport is disrupted.

In woodchucks, the drug lowered viremia, in some cases to undetectable levels, he said. At the same time, host glycoproteins appeared almost unaffected, implying that the drug is highly selective for the viral proteins.

Block cautioned that it is difficult to tell if the reduction in viremia will translate into a therapeutic benefit once the drug is used in humans. He said the research team hopes that longer-range studies will show N-nonyl-DNJ has therapeutic value. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 4 May 1998)

Hope for hepatitis

Researchers at the Liber Company in Tokyo are developing a drug which, they claim, could extend the life expectancy of hepatitis C sufferers. Currently, there is no effective treatment and the disease is inevitably fatal.

Hepatitic C is a cancer-causing virus. It triggers cancer by first creating what are known as "pre-cancerous lesions" throughout the liver. When a lesion develops into a full-blown tumour it can be removed by surgery or chemotherapy. But the chemotherapy needed to remove all the lesions would have such severe side effects that it would simply not be an option.

However, the team's current project seems to offer some hope. The researchers have pinpointed the active ingredient reinoid, a complex organic acid. It seems to act on both the pre-cancerous lesions and any developing tumours, but has no effect on non-cancerous, healthy cells, and so does not generate the debilitating side effects typical of most anticancer drugs, the researchers say.

In tests on cultured cells, the molecule seemed to induce abnormal cells to undergo apoptosis—a form of self-destruction. This not only worked with cells from hepatitis C-induced liver cancers, but also with cervical, lung and breast cancer cells.

The team also tested the drug on 44 volunteers with hepatitis C. Twelve of them suffered recurrent tumours, compared with 22 in a similar-sized group who received a placebo. (Source: *Chemistry & Industry*, 6 July 1998)

Joint action

The crippling symptoms of arthritis could be quelled by simple genetic therapy.

A growth factor called TGF-β is known to reduce the swelling of arthritic joints, but injections of concentrated TGF-β cause kidney damage, anaemia and other side effects.

Instead, Sharon Wahl of the National Institute of Dental Research near Washington, D.C. and her colleagues injected the TGF-β gene into the thighs of rats with arthritic symptoms so that a small amount of the protein was continuously released into the blood.

The paws of these rats swelled to only 4 per cent more than normal size, compared to 68 per cent for untreated animals. The effects of an injection lasted up to three months with no negative side effects. (Source: *New Scientist*, 27 June 1998)

Joint implants could last decades if disguised as bone

By anchoring metal to bone more effectively, materials scientists hope to produce an artificial joint that lasts for decades instead of a few years.

As new bone grows around an implant a gap tends to appear, so that eventually the implant works loose. As a result, the useful life of am implant may be less than ten years.

In the past, orthopaedic specialists have tried to make titanium joints look more like real bone by coating them with

hydroxyapatite, the hard, white form of calcium phosphate that gives normal bone its strength. But while such coatings blend well with bone, they remain poorly attached to the implant.

Now Allison Campbell, a materials scientist at the Pacific Northwest National Laboratory in Richland, WA, thinks she may have solved the problem. Before applying the hydroxyapatite, she prepares the metal by linking a long-chain carbon molecule to the titanium atoms via a bridge of silicon and oxygen. She then immerses the metal in a solution of carboxylic or sulphonic acid, molecules that serve as attachment points for the hydroxyapatite. The final soaking is a solution of calcium and phosphate, the components of hydroxyapatite.

Campbell and her colleagues place coated titanium in contact with bone cells in a culture dish. Bone grows by a process called remodelling, in which cells called osteoclasts break down old bone, while others called osteoblasts secrete collagen and hydroxyapatite to make new bone. Campbell says the bone cells began remodelling the hydroxyapatite coating around the metal, replacing it with fresh bone.

The relatively mild conditions of the anchoring procedure make it possible to add growth factors to the mix. Campbell is now testing TGF-β, a peptide that stimulates cell division. (Source: *New Scientist*, 27 June 1998)

Drugs hit the spot

A star-shaped polymer developed at Purdue University, IA, could deliver high concentrations of drugs to tumours, or even remove cholesterol from the blood. Chemical engineer Kelley Keys told the Controlled Release Society meeting in Las Vegas that she has created a polymer whose molecules have a central core with many arms radiating from it. The end of each arm can be highly chemically reactive, allowing it to bind with cells, drugs, proteins and antibodies, she says. The star molecules are only one hundredth the size of a red blood cell. (Source: *New Scientist*, 4 July 1998)

Syphilis cocktail

A vaccine for syphilis may soon be developed now that biologists have sequenced the entire genome of *Treponema pallidum*, the bacterium that causes the sexually-transmitted disease.

Combating syphilis is particularly important because the genital ulcers it causes help to spread HIV.

Researchers report that *T. pallidum* possesses 22 genes for proteins that may appear on its surface. Purified cocktails of any of these proteins may be effective as a vaccine against the disease, says lead author Claire Fraser of the Institute for Genomic Research in Rockville, MD. "All of them are immediate candidates for vaccine development." (Source: *New Scientist*, 25 July 1998)

Resistance is futile

An antibiotic used as a last resort when others have failed has been synthesized for the first time. Scientists hope the synthetic version of vancomycin will lead them to new antibiotics to fight resistant strains of bacteria.

Vancomycin is currently prepared from cultures of *Streptomyces* bacteria, which produce it naturally. But now David Evans of Harvard University, MA, and K. C. Nicolaou of the Scripps Research Institute in La Jolla, CA, have independently found ways to synthesize the drug.

The methods will not immediately boost supplies of vancomycin, as they are as yet too laborious for commercial production, but they could allow scientists to make slightly altered versions of the drug, which could be effective against emerging antibiotic-resistant bacteria. (Source: *New Scientist*, 10 October 1998)

Lab-grown bladders work in animals, seen as vision of medicine's future

In work that one scientist called a vision of the future of medicine, researchers used the cells from a dog's excised bladder to grow a new organ which was then implanted into the animal where it worked just as well as the original bladder.

The research team at Children's Hospital in Boston was able to perform the experiment successfully in six consecutive trials.

Other researchers in Germany showed they were able to do the same type of experiment in rats.

Dr. Anthony Atala, M.D., a researcher at Children's Hospital and Harvard Medical School, said the success in animals means that clinical trials could begin within two years in humans who lose their bladders due to disease or trauma.

Atala said that in a series of experiments the bladders of 14 beagles were excised, leaving only the bladder neck in the animals. In two dogs, no further intervention was done in order to see if the dogs would regenerate a bladder spontaneously. They did not.

In six other dogs, a polymer matrix in the shape of a bladder was implanted in the beagles to see if cells would grow around the structure. Atala said there was some infiltration of cells but generally the matrix failed to take on characteristics of a normal bladder.

The dogs without bladders were able to retain just 22 per cent of the normal capacity; the dogs with the surgically implanted matrix were only able to function at 46 per cent of normal capacity.

In the other six beagles, cells from the original healthy bladder were regrown in the laboratory. Those cells were fashioned about a polymer matrix and when the matrix was covered with cells, the bladder formed outside the body was transplanted into the dog.

The tissue-engineered bladders were working at 80 per cent efficiency after four weeks, and the average bladder performed at 95 per cent efficiency. Atala said people become aware of bladder insufficiency when the organ operates at less than 50 per cent of capacity.

Atala said the new organs developed normal blood vessels and regrew bladder nerves as well.

Doctors from Germany and San Francisco reported similar success in engineering rat bladders *in vitro* and then re-implanting them in the host animals.

Atala predicted that human trials for patients who face loss of the bladder could begin by the end of the century. (Source: *McGraw Hill's Biotechnology Newswatch*, 15 June 1998)

Test can tell if prostate cancer has spread from gland to bones

A new test can determine if prostate cancer cells have spread from the gland to bones, said researchers at the American Urological Association meeting in San Diego.

Dr. Michael Brawer, director of the Northwest Prostate Institute of Northwest Hospital, Seattle, said the test measures serum N-telopeptide, a substance created when osteoclasts break down bone.

N-telopeptide can occur when any cancer metastasizes to bone and begins destruction of the bone.

"Prostate cancer most often metastasizes to the bone", said Brawer, which means the N-telopeptide test could give doctors additional information in how they can treat prostate cancer in individual cases. Once prostate cancer metastasizes the chance of controlling it is greatly reduced. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 15 June 1998)

The nose could transform the treatment of brain diseases

Alzheimer's disease and other neuro-degenerative disorders could soon be treated with nose drops. Researchers in Minnesota say that the nasal passage holds great promise as a conduit for delivering drugs into the brain, as the olfactory system provides a direct link between the brain and the outside world.

The molecules of many drugs are too large to cross the blood-brain barrier, however nerve growth factor, a promising treatment for Alzheimer's disease, is one such drug. Injecting NGF does not work, and procedures such as grafting NGF-producing cells directly into the brain are expensive and risky.

Olfactory nerves are unusual in that they run straight from the olfactory bulb in the brain to the nasal cavity, where they come into contact with "odorants" in the air. But occasionally, the odd anatomy of the olfactory system can be dangerous because it provides a way for some viruses, such as herpes, to get into the brain. In one study, even tiny gold particles sprayed into the noses of monkeys were traced along the olfactory nerves and into the animals' brains.

William Frey, a neuroscientists at the Alzheimer's Research Center at the Regions Hospital in St. Paul and his colleagues decided to see if NGF could be delivered in nose drops. After anaesthetizing 12 rats, the researchers gave half the animals drops in their noses over 30 minutes and injected the rest with NGF. They found that within an hour, a significant amount of NGF had made its way not only into the olfactory bulb, but also into the hippocampus, amygdala and other regions not directly involved in smelling. In contrast, very little of the NGF injected into the other rats reached the brain. The same results held true when the researchers administered insulin growth factor 1, a possible treatment for stroke, in nose drops.

Frey's team says the nose could deliver drugs not only for Alzheimer's disease but for a range of other neuro-degenerative conditions as well, including Parkinson's disease and multiple sclerosis. The team has a patent on the idea and is working with a biotechnology company to develop it. (Source: *New Scientist*, 5 September 1998)

Thalidomide cleared for leprosy

Thalidomide, the drug best known for causing horrible birth defects in thousands of babies about four decades ago, has been approved for the market as a treatment for leprosy.

Celgene Corp., of Warren, NJ, has said it received clearance from the US Food and Drug Administration to market and sell Thalomide (thalidomide) for the treatment of *erythema nodosum leprosum*, a painful inflammatory complication of leprosy that causes bumps under the skin.

Celgene licensed rights to thalidomide, an angio-genesis inhibitor, from The Rockefeller University in 1992 and began developing the drug for a range of diseases, including conditions of the skin, AIDS and cancer. (Source: *McGraw Hill's Biotechnology Newswatch*, 20 July 1998)

Vical to test cancer vaccine

Vical Inc. has started Phase I/II trials of vaccine to fight metastatic melanoma, under the direction of cancer immunology pioneer Steven A. Rosenberg, M.D., Ph.D., Chief of Surgery of the National Cancer Institute, which is sponsoring the study.

The experimental vaccine contains a gene designed to cause cells at the injection site to produce a modified gp100 melanoma antigen, which will trigger an immune response against melanoma tumour cells.

The first stage of the trial will enrol up to 16 patients in each of two groups to determine whether intramuscular or intradermal administration is more effective.

The second stage will use the more effective administration method in treating up to 33 additional patients. Patients in the second stage will also be treated by intravenous administration with IL-2. (Source: *McGraw Hill's Biotechnology Newswatch*, 20 July 1998)

Antisense starts making sense

The first drug based on antisense technology—a way of preventing specific proteins from being made—may soon go on sale. The US Food and Drug Administration looks set to give the drug the go-ahead after an FDA advisory committee recommended its approval.

The drug combats an eye disease that is common in people with AIDS. Known as CMV retinitis, it is caused by the cytomegalovirus, a herpes virus. The drug blocks the production of a protein that the virus needs to replicate.

Antisense drugs are designed to block the production of specific proteins at the genetic level. They consist of RNA-like molecules which are complementary to—and thus bind to—a specific portion of the mRNA molecules which carry the information needed to make the targeted protein. With this portion blocked by the drug, the offending protein is not made.

The new drug, called fomivirsen, or Vitravene, is made by Isis Pharmaceuticals of Carlsbad, CA. It is injected into the eye every few weeks. The market for the drug in the US could be worth $100 million a year. (Source: *New Scientist*, 1 August 1998)

Livestock applications

Bonnie baby

The birth of Dolly's first lamb, Bonnie, provides further reassurance that clones can develop into healthy animals, but researchers at the Roslin Institute near Edinburgh, who cloned Dolly, say it will take several months to show whether she and her offspring have escaped chromosome abnormalities that could make them die young.

Dolly's successful pregnancy is good news for those who want to commercialize animal cloning.

While mammalian clones have given birth before, extra questions surrounded Dolly's fertility because she was cloned from a cell of a six-year-old adult—by far the oldest "parent" of any clone.

One hallmark of mammalian ageing is the steady loss of sections of DNA, called telomeres, from the ends of chromosomes. Some scientists have suggested that because Dolly was derived from an old cell, she might have unusually short telomeres. (Source: *New Scientist*, 2 May 1998)

Test to identify Salmonella-resistant chickens

Poultry breeders could soon have access to a genetic test which identifies chickens that are resistant to *Salmonella* bacteria.

Nat Bumstead and his colleagues at Britain's Institute for Animal Health in Compton, Berkshire, discovered a gene linked to resistance by analysing DNA from crosses between resistant and susceptible chickens. The institute is now developing a test for the gene which would enable chicken breeders to supply farmers with entire flocks that are resistant to *Salmonella*, limiting contamination of eggs and carcasses.

"It means we can begin to reduce the load of *Salmonella* that comes off the farm in the first place", says a spokesman for the institute. (Source: *New Scientist*, 11 July 1998)

Which prion?

A new antibody test can distinguish one strain of prion disease from another, say researchers in San Francisco. So far the test has been shown to work with hamsters infected with scrapie, which normally affects sheep. Eventually it might reveal whether BSE has crossed from cows into sheep, as some researchers fear it may have done.

Current techniques for identifying strains of prion disease are very slow. Tissue must be injected into mice and left to incubate for a year or more. Only in this way can BSE be distinguished from the various strains of scrapie, each of which has a unique delay from infection to the onset of symptoms. Now a team at the University of California, San Francisco, led by the Nobel prizewinner Stanley Prusiner, has devised a rapid test that can distinguish between eight strains of scrapie.

Prions, which are a rogue version of the protein PrP, form clumps to which antibodies against normal PrP do not easily bind. But by adding a chemical called guanidinium to brain samples taken from infected hamsters, the researchers partly broke up the clumps, allowing antibodies to bind. They found that the ratio of the quantity of antibodies binding to the PrP before and after treatment with guanidinium was unique to each strain. (Source: *New Scientist*, 3 October 1998)

Agricultural applications

Transgenic insect-resistant crops harm beneficial insects

Two recent European studies report troubling, and unexpected, effects of genetically engineered insect-resistant crops on beneficial insects—green lacewings and ladybird beetles.

Scientists from the Swiss Federal Research Station for Agroecology and Agriculture looked at the indirect effects of Bt corn on green lacewing insects that feed on the European corn borer (ECB), the pest targeted by Bt corn. They compared the mortality and developmental rate of two groups of lacewings—one that had been fed ECB reared on engineered Bt corn and another fed ECB reared on non-Bt corn.

The experiments revealed that green lacewings fed ECB that had eaten Bt corn had a higher death rate and delayed development compared with lacewings fed ECB that had eaten non-Bt corn. More than 60 per cent of the lacewings fed Bt-corn-reared ECB died compared with fewer than 40 per cent of those fed on non-Bt-corn-reared ECB. The researchers suggest that the higher mortality is "directly associated with [Bt]-related factors". Among surviving lacewings, those feeding on Bt-corn-reared ECB required an average of three more days to reach adulthood than lacewings fed on non-Bt-corn-reared ECB. The scientists attributed the delayed development to the poor nutritional quality of ECB larvae made sick by the Bt toxin.

In other study, Scottish Crop Research Institute scientists found that ladybird beetles fed aphids reared on transgenic potatoes experienced reproductive problems and failed to live as long as ladybirds fed aphids from ordinary potatoes (the control group). The potatoes were engineered to produce insecticidal lectins—proteins from the snowdrop plant that bind to the surface of insect cells causing them to clump and stop functioning. In greenhouse tests, the engineered plants significantly reduced potato aphids—a serious pest of the crop—compared with non-transgenic ones.

The researchers found that the egg production of female ladybirds fed transgenic-potato-reared aphids was reduced by more than one third, compared with the control group. Matings between male ladybirds fed on aphids from transgenic potatoes with females from the control group produced four times as many unfertilized eggs as matings with males from the control group. Nearly three times as many fertilized eggs from females fed engineered-potato-reared aphids died before hatching compared with fertilized eggs from the control group. Finally, female ladybirds fed aphids from transgenic plants lived only half as long as females from the control group.

Neither of the studies has been extended to field situations so it is far from clear whether these laboratory results reflect what might happen outdoors. But field results show similar deleterious effects on survival, reproduction and development, then large-scale use of transgenic insect-resistant plants could be expected to diminish populations of beneficial insects. (Sources: A. Birch et al., "Interactions between plant resistance genes, pest aphid populations and beneficial aphid predators", 1996/97 Scottish Crop Res. Inst. Annual Report, Dundee, pp. 68-72; A. Hilbeck et al., "Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae)", *Environmental Entomology* 27: 480-87, 1998) (Source: *The Gene Exchange*, Summer 1998)

New late blight resistant potato varieties

New kinds of potato that resist late blight could help farmers avoid billions of dollars of damage to their crops annually, according to the International Potato Centre in Lima, Peru. The centre announced that it will begin releasing resistant varieties to breeders, so farmers could be growing the potatoes within a few years.

Late blight, caused by the fungus *Phytophthora infestans*, was responsible for the Irish potato famine 150 years ago. In recent years more virulent strains of blight have appeared, cutting harvests worldwide by 15 per cent and costing about $3.25 billion in lost yields.

The genes responsible for making the new varieties resistant were found in a wild potato subspecies from Peru, held in the International Potato Centre's collection. The subspecies, *Solanum tuberosum andigena*, has been crossed with other potatoes, producing about 60 new varieties with the combination of disease resistance and high yield needed by farmers.

The new plants should be much more robust because their resistance comes from 10 to 20 different genes. Even if the blight finds a way around one or two of the genes, it will still be defeated by the others.

The International Potato Centre, funded by the Consultative Group on International Agricultural Research, a non-profit-making body, does not develop new varieties for sale, but commercial breeders will be given access to the new potatoes so that they can develop varieties that are tailored for different climates and tastes. (Source: *New Scientist*, 30 May 1998)

Cereal killer targets potato blight

A chance discovery has revealed that slurries of malted wheat, barley or rye can kill the organisms that cause two devastating diseases in potatoes—late blight and soft rot.

Karen Barrett of the Idaho National Engineering and Environmental Laboratory in Idaho Falls made her discovery while studying biofilters—communities of micro-organisms that generate atmospheric nitrogen from gaseous nitrogenous compounds in industrial waste streams. The crucial moment came while she was looking for slow-release foods that would sustain the colonies during weekends, when she was away from the laboratory.

Barrett tried cereals because they contained all the right nutrients and a nearby brewery could provide a convenient supply. One Friday she loaded the biofilter container with malted, or germinated, grains, but when she returned on Monday the colony was dead. When she rebuilt the biofilter leaving out the malted cereals, the micro-organisms thrived.

Alexander Fleming used similar reasoning about the mould that killed his staphylococcus culture—the inspiration for the discovery of penicillin in 1928.

Barrett tested slurries against the bacteria that cause soft rot in potatoes. They all worked. She also tried the slurries against late blight, which led to the Irish potato famine of the 1840s. Late blight is now becoming more common throughout the US, thanks to mutant strains that are resistant to fungicides. Barrett's slurries killed off one aggressive strain known as genotype US-8.

Barrett is now working to find out which of the compounds released when seeds germinate are actually killing the microorganisms. (Source: *New Scientist*, 8 August 1999)

New method to propagate potato plants

Agriculture and Agri-Food Canada's Fredericton Research Centre in New Brunswick has developed a new way to propagate potato plants. The method, which produces somatic embryos *in vitro* on potato tissue, has been effective on 18 cultivars. It is believed that the technique can be used for genetic modification purposes, including the production of vaccines and synthetic seeds. The centre is now looking for commercial partners to collaborate on further development.

Contact: Gilles Saindon by tel.: (506) 452-4831; fax (506) 452-3316; e-mail: saindon@em.agr.ca. (Source: *The AgBiotech Bulletin*, January 1998)

Rice paddy field herbicide using microbes

Mitsui Chemicals Inc. has developed a rice paddy field herbicide using micro-organisms that is selectively effective for barnyard grass, the principal grass obstructing rice plant growth. The herbicide effect has been confirmed through corporate tests and will be placed on the market in the year 2002.

More than 7,000 strains of fungi (moulds) belonging to the Fusarium, Poma and Drechslera families were isolated from the tissue of barnyard grass growing wild. The results of tests of the herbicidal effects of these strains using barnyard grass and rice plant led to the identification of the *Drechslera monoceras* MTB-951 strain that displays a strong selective herbicidal effect with respect to barnyard grass. This strain displays a herbicidal effect with respect to tainubie and all *Echinochloa* genus species, but no pathogenic symptoms were recognized with respect to rice plant species.

A powder herbicidal agent was prepared which consisted primarily of the conidia of this cultured fungal strain, and was tested as the early-stage herbicide in the direct sowing and cultivation of rice plants. The results corroborated that over 90 per cent of barnyard grass was killed off without any adverse influence even on newly sprouted rice plants.

When the fungal strain conidia sprouts on barnyard grass, pathological spots appear in about a week and the grass withers in about 10 days. Since this herbicide acts only on barnyard grass, there is no fear of rice plants being damaged, and there is also the advantage that it does not remain as residue in the soil.

The micro-organism displaying safe herbicidal effect was discovered after searching throughout the country. The mechanism from barnyard grass infection to its withering is as yet unclear, but a concentration of about 30 billion micro-organisms will provide highly satisfactory results per 10 acres. The herbicide will be manufactured in granular form, so dissolving in water, followed by spraying, which appears to be the ideal method of use.

A two-stage culturing process has been established for culturing the micro-organism and exposing the conidia in the open air for its proliferation. The company plans further study of the herbicide to lower its production cost to a level comparable to those of agricultural chemicals, and plans to establish some methods for other agents in combination for applications other than coping with barnyard grass. Further details from Mitsui Chemicals Inc., Public Relations Department, 3-2-5, Kasumigaseki, Chiyoda-ku, Tokyo 100-6070. Tel.: +81-3-3592-4105; fax: +81-3-3592-4211. (Source: *JETRO*, October 1998)

New satsuma mandarin culturing technique permits rapid harvesting

The Aichi Prefectural Agricultural Research Center has established a new satsuma mandarin culturing technology that enables the fruit to be harvested in one half the number of years normally required by conventional culturing techniques. Nursery stocks cultured inside synthetic fibre containers are transplanted into a greenhouse by which these plants bear fruits in as little as three to four years.

The Rapid Growing Method for Nursery Stocks is essentially a technique to produce nursery stocks rapidly to gain yields with stability in short periods of time. The plant roots are concentrated along the fringes of their containers. So the one-year nursery stocks are planted in 20-25 litre non-woven fabric containers, which prevent root coiling that obstructs rapid plant growth, then cultured in a greenhouse, by which large nursery stocks are obtained in one year and four months. This is much quicker than the ordinary open culturing method that usually requires six years.

The use of soft non-woven cloth containers prevents the roots from being injured when planting these nursery stocks, so transplanting is accomplished smoothly and efficiently. The nursery stocks are cultured densely inside a greenhouse by the extra-dense culturing method, acquiring the same volume of yield as an ordinary hothouse culturing facility. To ease ancillary work, the trees are grown only to a height of about 2 metres, by which substantial labour-saving is possible through hedge-row training.

A blossoming stabilization technique is also introduced of covering the soil surface with a white sheet of excellent light reflection, to supply sunlight to the dark lower parts of the tree canopy. This was confirmed to generate buds at the lower parts of the tree canopy, and also prevent the fall of premature fruits. An experiment is also being advanced on an alternate year fruit-bearing culturing method of acquiring a yield of 1.4 volume in one alternate year, and of conserving the heating cost and vinyl covering cost. Further details from Aichi Prefectural Agricultural Research Center, Planning Information Department, 1-1, Sagamine, Yazako, Nagakute-cho, Aichi-gun, Aichi Pref. 480-1193. Tel.: +81-561-62-0085; fax: +81-561-63-0815. (Source: *JETRO,* October 1998)

Mass propagation of dipterocarpaceae trees by tissue culture

Sumitomo Forestry Co., Ltd. has established a technology for the mass propagation of the dipterocarpaceae tree, a leading type of tropical rain forest tree, by tissue culture. The seed of this tree is difficult to acquire, and cuttings are also difficult to root from a mature tree. The new technology allows mass propagation of the tree repeatedly and rapidly by tissue culture. There is no known technology for the mass propagation of the dipterocarpaceae tree, so the new technology raises the possibility of considerably promoting the reforestation of tropical rain forests in the south-east Asian region.

Tissue culture was performed successfully with the *Shorea roxburghii*, an indigenous tropical rain forest tree that grows to a height of over 40 metres and belongs to the dipterocarpaceae family.

The upper part of the tree bud was used for tissue culture. The shoot tip was cultured in a medium containing two types of saccharides, and multiple shoots consisting of many buds were generated in about three months. The multiple shoots are then transplanted in a new liquid medium that permits long-term culture, and cultured with a rotary machine. The grown shoots are excised one by one, then transplanted into a vermiculite solid medium to promote rooting. Roots grow out from the shoots when cultured for about one month after transplant, and the shoots grow into complete plants.

These plants are then transplanted into pots and acclimatized to the natural environment. When using shoots grown from auxiliary buds, the rooting ratio is about 20 per cent, but shoots grown from the multiple shoots were rooted successfully by virtually 100 per cent. In general, when the dipterocarpaceae tree is grown by cutting a branch, the tree tends to grow slantwise due to the branch characteristics, making it difficult to acquire proper saplings. However, applying the new technology enables saplings to be produced en masse from the shoot tips of any heart of the tree. Further details from Sumitomo Forestry Co., Ltd., Tsukuba Research Institute, 3-2, Midorigahara, Tsukuba City, Ibaraki Pref. 300-2646. Tel.: +81-298-47-0153; fax: +81-298-47-0156. (Source: *JETRO*, October 1998)

Electron bombardment

For a number of years, scientists at the Fraunhofer Institute for Electron Radiation and Plasma Technology in Dresden have had their sights set on pathogens in cereal seed stocks. They have now developed a technique which removes pathogens from seeds in an environmentally friendly, chemical-free, and inexpensive way. The researchers send tons of grain through a radiation installation where they are gently bombarded with low-energy electrons. This is an effective way of eliminating pests on and in the seed coat. The energy of the electrons can be precisely controlled so that the electrically charged particles only penetrate the outer shell of the seeds without damaging the plant embryo. (Source: *Deutschland*, No. 3, June 1998)

Spent force

Spraying crops with proteins from the brains of insect pests could deter the bugs by wrecking their reproductive cycles, say researchers in Texas. The idea could lead to a new and safer way of controlling pests.

Brain cells signal to one another with neuropeptides, short proteins that bind to receptors on the cell's surface. A neuropeptide in the brain of the corn earworm, *Helicoverpa zea*, a common pest of the cereal crop maize, controls the production of pheromones, which are secreted by the female earworm moth to attract mates.

Ronald Nachman and his team at the US Department of Agriculture's research centre in College Station, Texas, hoped that an excess of this peptide would disrupt the adult earworm's mating patterns. To make the peptide easier to handle, they shortened it from 33 amino acids to only five. The short version still worked when injected into the moth, although it was less potent.

To make an effective spray, they had to modify their peptide fragment so that it would penetrate the moth's hard exoskeleton or cuticle. So they replaced one amino acid with a boron-rich, ball-shaped molecule called carborane. The resulting peptide not only slid through the insect's cuticle, but also stimulated pheromone production 10 times more powerfully than the naturally occurring peptide. The researchers believe this occurs because the carborane protects the peptide from enzymes that might normally break it down.

Moth treated with the phoney peptide actually consumed their entire lifetime's store of the raw materials needed to make the sex pheromone. So in theory, a moth emerging from its pupa in a field sprayed with the peptide would use up all its pheromones before it reached sexual maturity—and would not be able to breed.

Nachman says human toxicity studies and field tests will take several years, but he expects that the peptide will not be toxic to other animals or beneficial insects. (Source: *New Scientist*, 23 May 1998)

Papaya approved

Genetically engineered papaya has been approved for marketing in the US, marking the first commercialization of a transgenic virus-resistant perennial fruit crop. The transgenic plant, which resists papaya ringspot virus (PRV) infection by producing a portion of the PRV coat protein, took researchers from Cornell University (New York), University of Hawaii, and Upjohn (Kalamazoo, MI) 10 years to develop. The two lines of PRV-resistant papaya, SunUp and Rainbow, were approved in 1997. Their last commercialization hurdle was crossed this April when the Cornell Research Foundation and the Papaya Administrative Committee (PAC; Hilo, HI) obtained licences to use the genetic technology from Monsanto (St. Louis, MO), MIT, and others, says Dennis Gonsalves, a Cornell plant pathologist who led the team. According to the USDA Foreign Agricultural Service, most of the US papaya export, which accounts for 8 per cent of worldwide volume, comes from Hawaii. However, PRV causes poor quality, ring-spot blemished papaya fruits, eventually killing the plant. Papaya production in Hawaii has

progressively decreased over the past four years, seriously damaging its $45 million industry. (Source: *Nature Biotechnology*, Vol. 16, June 1998)

Growing for gold

Prospectors may one day be cultivating crops instead of panning for gold, thanks to New Zealand scientists who have discovered how to make plants soak up large amounts of the precious metal from the soil.

The researchers, from Massey University in Palmerston North, say their technique could make "biological mining" for gold and other precious metals commercially viable for the first time.

Plants are already used to collect valuable heavy metals such as nickel from contaminated soil, but gold is absorbed in such small quantities that it cannot be collected commercially, even from soil near gold mines.

The new technique increases absorption by a factor of thousands. Robert Brooks and colleagues at the university's department of soil science found that Indian mustard plants (*Brassica juncea*) readily absorbed gold from a solution of ammonium thiocyanate, a substance used in mining to make gold soluble.

Plants grown on soil treated with ammonium thiocyanate also absorbed gold. Depending on the conditions, concentrations of four parts per million (ppm) could be increased to as much as 57 ppm, the researchers report. The average was about 10 ppm. At today's gold prices of $300 per ounce the commercial threshold is about 17 ppm.

The thiocyanate kills the plants after about a week, but Brooks says this is not a problem because Indian mustard grows very quickly. The gold can be retrieved by burning the dead plants and refining the ash.

The researchers now hope to develop an experimental plot at a gold mining site in New Zealand. They say that chicory, which grows well near mines, could be used instead of Indian mustard.

Although applying ammonium thiocyanate to soil costs $3,627 a hectare, commercial biomining operations could recoup the cost by selling the energy from burning dead plant material, the researchers suggest. (Source: *Chemistry and Industry*, 19 October 1998)

Genetically engineered crops may produce hard-to-kill weeds

According to an Ohio State University study, weeds that had acquired genes for herbicide resistance from genetically-altered crops were able to reproduce as easily as unaltered weeds. Scientists have known for years that transgenic crops, such as those engineered to be herbicide tolerant, can pass their traits onto nearby weeds via hybridization. These hybrid, transgenic weeds resist the herbicides that were designed to kill them. However, scientists also hypothesized that hybridization might cause some negative characteristics to emerge in a weed that would limit its reproduction. For example, a hybrid weed might produce fewer flowers or seeds than a pure weed.

Allison Snow, associate professor of plant biology at Ohio State collaborated with Risoe National Laboratory in Denmark to find out whether this was the case for oilseed rape. The study suggests that, at least in the case of oilseed rape, weeds that cross with commercial crops and acquire a specialized transgene will be able to reproduce with few if any problems. The researchers presented their results at the 1998 Ecological Society of America Annual Meeting.

Snow and her colleagues crossed a herbicide-resistant version of oilseed rape with a weedy cousin and cultivated their progeny in indoor growth chambers in Denmark. They found that even when the unaltered weeds were given the advantage of an ideal growing environment, they did not on average produce more fruits or seeds than the hybrid weeds. While initial generations of altered weeds looked different, by the third generation, weeds that carried the gene looked exactly like normal weeds. Snow stated that the only way farmers would be able to tell the difference would be to spray all the weeds with herbicide and see which ones survive. Previous studies have shown that oilseed rape pollen can reach weeds nearly one mile away.

Source: "Genetically-altered Crops Can Produce Tough, Hard-to-Kill Weeds", Ohio State University press release, 6 August 1998. (Source: *Global Pesticide Campaigner*, September 1998)

A first step towards engineering improved phosphate uptake

Among the trio of major plant nutrients, phosphorus is the most limiting compared to nitrogen and potassium. Many soils are low in phosphorus and even when it is abundant, uptake of this nutrient by plants can be tricky. Bioavailability of phosphorus is very critical in the acidic soils of the tropics where iron and aluminium interfere with its uptake. Thus, millions of acres of land in developing countries have phosphorus deficiency problems. Calcium-rich soils in the south-east and Great Plains of the United States are also plagued with a similar problem. Countries such as India spend an enormous amount of their precious foreign exchange on importing phosphate fertilizer which is derived from rock phosphate found in a few areas such as the US, Russia, Morocco and Tunisia. Global reserves of high quality rock phosphate are limited and may run out in about 100 years according to one estimate.

A logical way to address this problem is by developing plants which can efficiently draw phosphate (a common form of phosphorus) from soil. Until recently, scientists knew very little about the molecular basis of how plants absorb critical nutrients such as phosphorus, potassium and sulphur. A recent flurry of papers reporting the isolation of ion-transporters in plants is improving our understanding of nutrient uptake in plants. A research group led by K. G. Raghothama at Purdue University were the first to clone phosphate transporter genes in plants from *Arabidopsis* in 1996.[1] More recently, they have also shown the existence of such genes in tomato.[2]

To clone the phosphate transporter genes, the Purdue group grew roots of *Arabidopsis* under phosphate-deprived conditions. This led the plants to switch on the phosphate transporter genes which were then isolated using expressed sequence tags (ESTs) as probes to screen a plant cDNA library. These genes were found to be expressed differentially in the roots of phosphate-starved plants. The phosphate transporter genes in tomato isolated by the Purdue group, and those from potato, *Medicago* and *Catharanthus* identified by other researchers, appear to be similar in structure and function to the *Arabidopsis* genes. Predictably, expression of the tomato genes appears to be localized in the root epidermis, the site of phosphate uptake. Changes in the cellular concentration of phosphorus apparently induce the expression of these genes. Raghothama's team is now developing transgenic plants to over express transporter genes to test whether this would result in a higher efficiency uptake of phosphorus.

Identification of genes involved in phosphate uptake is a major first step towards the eventual development of plants which can absorb phosphorus from soil in an efficient manner. Strategies like this may play an increasingly important role in the future to deal with the problems of poor soil fertility and to reduce the dependency on fertilizer application. This would be particularly welcome by resource-poor farmers in developing countries.

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Contributed by C. S. Prakash, Center for Plant Biotechnology Research, Tuskegee University from ISB News Report, May 1998.
(Source: *Australasian Biotechnology*, Vol. 8, No. 3, June 1998)

EPA nears approval for fire blight antibiotic

For the first time in 30 years, a new antibiotic may become available for controlling a bacterial blight that attacks apples and pears. AgryGent, the trade name for gentamicin sulphate, is moving through the Environmental Protection Agency's registration process.

AgryGent is already registered in Central and South America, and its manufacturer, Quimica Agronomica of Mexico, has registrations pending in 22 other countries.

The product is used in apple and pear orchards during the bloom period, a critical time for fire blight, a disease caused by a bacterial plant pathogen.

The disease is now spreading worldwide, particularly throughout the Mediterranean. Fire blight causes extensive crop losses and tree deaths, making it one of the most devastating plant pathogens.

Roberta Spitko, a plant pathologist with New England Fruit Consultants, Montague, MA, says that despite years of study, efforts to contain the disease often fail and it must be managed through multiple control strategies.

Quimica Agronomica has sought the registration of AgryGent for fire blight management since 1994. Only one antibiotic, streptomycin, is available for the disease. (Source: *Chemical Manufacturing Reporter*, 24 August 1998)

Telling the wood from the trees

Gene mapping is useful against all sorts of pests, including tree thieves. Wood rustling is easy and profitable. In British Columbia, for example, illegal felling of species such as the western red cedar is a growing problem. The provincial government also loses tax revenues; tree theft is thought to cost it as much as C$20 million a year. So Eleanor White, a researcher at the Canadian Forest Service in Victoria, British Columbia, has borrowed from forensic science and devised a way to use genetic markers to "fingerprint" valuable trees so that they can be traced if they are stolen.

To do this, Dr. White has developed a technique to extract DNA from dead wood using a mixture of special solvents. She then proceeds much as the police do in a forensic laboratory, amplifying the retrieved DNA using a technology known as polymerase chain reaction, and then cutting it up into millions of bits. These bits can be individually separated and probed for short stretches of repetitive DNA called microsatellites.

Each red cedar has a slightly different pattern of microsatellites. Dr. White has identified 10 microsatellite markers that can be used to build up a unique genetic profile of the individual tree. This means that sleuths will be able to compare the microsatellites in a DNA sample from suspicious lumber to those in what is left of the original tree in the woods (whose whereabouts in the province's vast forests can also be determined by genetic markers).

The fingerprinting technique is in final trials. It should be ready for use in catching thieves by the end of the year. The same method should be simple to extend to other bootlegged trees, such as the Douglas fir and white spruce. Jerry Hunter, an official at British Columbia's Ministry of Forests, suggests that the technology could be particularly useful for developing countries struggling with illegal timber exports. For instance, Cambodia's precious teak forests are rapidly and illegally making their way into European furniture.

Proving that wood is contraband is a crucial first step to prosecuting the thieves. (Source: *The Economist*, 25 June 1998)

Food production and processing

New vanilla flavouring

Bacteria could soon be manufacturing cheaper vanilla flavouring for ice creams, yoghurts and cakes if work by British researchers pays off. Their "biovanillin" could be the first of many valuable flavourings created in this way.

Authentic vanilla comes from vanillin, an extract of the pods of the tropical vanilla orchid, *Vanilla fragrans*. But demand exceeds supply for this expensive product and the food industry is seeking alternatives.

A team at the Institute of Food Research in Norwich says that a simple soil bacterium can make large amounts of vanillin from a cheap starting material found in agricultural waste.

IFR researchers Arjan Narbad and Mike Gasson used soil bacteria dug up near a green house at the institute. Their newly discovered strain of *Pseudomonas fluorescens* converts ferulic acid, found in agricultural waste, into vanillin.

They hope that biovanillin will be superior to synthetic vanillin, which is made from the plant fibre lignin or from eugenol, a substance found in clover oil. This synthetic vanillin accounts for 80 per cent of the 8,000 tonnes of vanillin consumed worldwide each year.

The researchers acknowledge that they cannot match the quality of the natural extracts, which can only be produced from the orchid pods. But because they have identified all the bacterial genes that control production of vanillin, they believe they can manipulate the genetic pathway so that the biovanillin includes additional flavourings found in the natural extract. These include para-hydroxy benzaldehyde and para-hydroxy benzoic acid, substances that the *Pseudomonas* bacteria could produce if they were fed with coumaric acid as well as ferulic acid.

To dispel fears about the safety of using soil bacteria to make edible substances, Narbad and Gasson plan to transfer the genes which manufacture vanillin from the *Pseudomonas* bacteria into organisms such as yeast or the bacteria that make

lactic acid, which are widely accepted as safe for use in the food industry.

They expect bacterial production to be cleaner than chemical synthesis, which generates sulphur dioxide. But they accept that they may face criticism from pressure groups opposed to developing processes which could undermine traditional vanilla production in poorer nations. (Source: *New Scientist*, 23 May 1998)

That cheese sounds great

Researchers in France have come up with an ultrasonic sensor that will listen to cheese as it matures and warn cheese makers of defects.

Cheese makers have to downgrade up to a fifth of their produce because of undetected faults. If the new device works well, that problem could be reduced.

The technique involves sending a low-frequency ultrasonic signal through the cheese to a sensor at the other side. By measuring the change in the speed and amplitude of the emerging signal, the fragmentation, moisture and porosity of the cheese can be mapped.

With ultrasound, cheese makers will be able to spot cracks in cheese that is only a day old. In response they will be able to adjust the temperature and the length of time that the cheese is left to mature.

Ultrasound could be a marked improvement over the conventional method of testing by inserting a cheese-iron. Ultrasound would leave the cheese intact and provide information more quickly. It also guarantees that the cheese remains clean, since no one need touch it.

So far the system has only been fully developed for one cheese, Comté, but it has been successfully tested on a variety of others. (Source: *New Scientist*, 1 August 1998)

Energy and environmental applications

Farmer's friend

Grasses that grow wild across Kenya can double yields of maize, Africa's most important grain crop. Trials in which the grasses were grown alongside maize have proved so successful that they are about to be repeated in three other African countries.

The grasses do battle with the stem borer, a caterpillar that decimates maize yields in millions of fields across eastern and southern Africa. When planted around the edges of fields, some species attract the moths that lay stem borer eggs, then secrete a gum that kills the cater pillars. They also attract the borer's worst foe, a parasitic wasp.

Other grasses, if planted between rows of maize, keep the moths away by giving off an unpleasant smell. The grasses also fend off a second major threat to maize crops, a weed called *Striga* that attaches itself to the plants' roots. Between them, *Striga* and stem borers typically cause a 40 per cent loss of maize.

Scientists at the research station at Mbita Point on the shores of Lake Victoria have tested four of these grass species—Sudan (*Sorghum vulgare*), napier (*Pennisetum purpureum*), silverleaf (*Desmodium uncinatum*) and molasses (*Melinis minutiflora*)—on 150 Kenyan farms. They proved so successful that the British-based Gatsby Charitable Foundation, which funded the research, is extending the programme to Uganda, Tanzania and Ethiopia. (Source: *New Scientist*, 24 October 1998)

NSF finds grazing produces biodiversity increase in grasslands

National Science Foundation (NSF) researchers have found that grazing by bison and other herbivorous mammals increases biodiversity in North American grasslands, even during periods of frequent burning and other stresses. In fact, loss of species diversity in these grasslands due to frequent burning was actually reversed by bison grazing, according to NSF ecologist Scott Collins.

The work was conducted at NSF's Konza Prairie Long-Term Ecological Research site in north-eastern Kansas, one of a network of 20 such NSF sites in North America and Antarctica.

In North American tallgrass prairies, diversity and productivity are controlled to a large extent by nitrogen availability, Collins explained. Historically, nitrogen availability in prairies was driven by interactions between frequency of fires and grazing by large herbivores. In general, spring fires enhance growth of certain grasses, and herbivores such as bison preferentially graze these grasses, keeping a system of checks and balances working properly, and allowing many plant species to flourish.

Collins and his colleagues conducted two long-term field experiments in native grasslands to assess effects of fire, addition of nitrogen, and grazing on plants species diversity. In one experiment, species richness declined on burned and fertilized areas, whereas grazing maintained diversity under these conditions. In a second experiment, loss of species diversity due to frequent burning was reversed by bison, animals that Collins calls keystone herbivores in North American grasslands.

"This research indicates that by adding or maintaining grazing", states Collins, "at least in ecosystems like grasslands that were impacted historically by these herbivores, diversity in native vegetation can be retained under conditions that would otherwise lead to a decline in species richness". For further information, contact: Scott Collins, National Science Foundation, 4201 Wilson Blvd., Arlington, VA 22230, USA. Tel: +1-703-306-1479. e-mail:<scollins@nsf.gov>. (Source: *Diversity*, Vol. 14, No. 1 and 2, 1998)

Ever green

A novel way of treating seeds with natural plant growth hormones could help restore Florida's Everglades to their former glory.

The cornerstone of this fragile ecosystem is a sedge called sawgrass, *Cladium jamaicense*, which once covered up to 80 per cent of the Everglades. Sawgrass normally spreads by rhizomes, and withstands both drought and flood. But excess nutrients from agricultural fertilizers and disturbance by boats have destroyed large areas of sawgrass. Because its seeds germinate poorly, faster-sprouting cattails, *Typha dominicans*, then take its place.

This threatens the entire ecosystem, says Charles Carraher of Florida Atlantic University in Boca Raton. Not only do cattails struggle to deal with extreme high or low water levels, but their roots also fail to support the layer of microscopic life called paraphytin that is the base of the Everglades food chain.

Spreading seeds from boats or planes would be the easiest way to regenerate sawgrass, says Carraher, if enough seeds could be made to sprout. To increase the germination rate, he synthesized polymers consisting of chains of

giverellin or kinetin growth hormone molecules, then mixed them with talcum powder to make them stick to the seeds. Adding tin to the polymers prevents fungal infections for up to three months.

The treatment can raise sawgrass seed germination rates up to 25-fold. Carraher hopes to begin trials in the Everglades within the next year or two. (Source: *New Scientist*, 5 September 1998)

Little helpers

It is easy to measure the damage that logging does to forests, but monitoring their recovery is expensive and hugely time-consuming.

Fortunately, conservationists may be able to rely on a cheap set of tools to help them: ants and bees. Ants are highly sensitive to the state of the soil, and bees are very particular about the species of tree they pollinate or nest in. So these insects alone give a comprehensive picture of the health of recovering forests, says a team of German and Malaysian researchers.

Karl Linsenmair, a biologist at the University of Würzburg is heading the research team studying how ant and bee communities respond to logging by looking at a range of environments, from undisturbed woodland to heavily logged land, in the 55,000-hectare Deramakot Forest Reserve in the Malaysian part of Borneo. The number of ant species in an ecosystem depends on the microclimate, soil structure and vegetation, all of which are influenced by logging techniques, which damage the forest floor and lead to erosion. The team believes that an analysis of ant communities will reveal any damage to a forest floor.

Damaged forest will have fewer suitable nesting sites for bees and the absence of certain bee species would suggest that the trees they prefer are depleted. (Source: *New Scientist*, 1 August 1998)

Growing pains

In Costa Rica, money grows on trees. Thanks to a deal struck by the Government, American and Norwegian businesses are about to pay farmers there to plant forests on their land. The farmers do not even have to produce timber—they just have to let the trees grow.

The companies will pay the farmers $10 for every tonne of carbon that their trees absorb from the atmosphere and convert to plant tissue during photosynthesis. In return, the companies will get certificates declaring that they have paid for CO_2 to be removed from the atmosphere, or "sequestered". They are hoping that these certificates, known as carbon credits, will soon become valuable commodities.

The deal will help fulfil the aims of the UN summit on climate change in Kyoto which set targets for industrialized countries to reduce their emissions of CO_2 and other so-called greenhouse gases. The Kyoto protocol allows countries to meet part of their targets by planting forests to soak up CO_2 instead of making cuts. It also sets the scene for nations and businesses to trade in carbon credits.

The Costa Rican Government and its partners, which include the Norwegian industrial giant ABB and a Chicago company called Environmental Financial Products, believe they are in at the start of a multibillion-dollar business.

Costa Rica has already sold credits for more than 200,000 tonnes of carbon. Many analysts believe that once the system is up and running, carbon credits could change hands for $100 a tonne or more. If so, the companies that are buying credits in Costa Rica today for $10 a tonne could make vast profits. "The price is right", says Richard Sandor, the chief executive of Environmental Financial Products and a long-standing advocate of trading in environmental resources.

But there is a host of practical problems with carbon sequestration. Most importantly, there is no way as yet to accurately measure how much carbon is absorbed or released by forests as they grow, die or burn.

John Lanchbery, who analysed the Kyoto agreement for the Verification Technology Information Centre in London, says it is inherently difficult to estimate how much CO_2 is produced or absorbed by biological activity. For instance, different species grow at different rates. And trees assimilate carbon fastest when they are young and slow down as they get older.

To complicate matters further, the growth rates of trees depend on the climate, itself made increasingly uncertain by global warming. A drought could drastically slow the uptake of CO_2, or even put it into reverse if the forest caught fire. Most of the carbon can be released from burning forests within a few days or weeks. And plant diseases, which themselves become unpredictable with climate change, can kill off vegetation, with similar effects. Nitrogen oxides in acid rain, for instance, have a considerable effect on a forest's capacity for absorbing carbon.

With no way of knowing exactly how much carbon trees take form the atmosphere, a reliable scheme to trade in carbon credits looks impossible. Yet new forests have a big potential as carbon sinks. In optimum conditions, tropical plantations can absorb up to 100 tonnes or more of CO_2 per hectare in 50 years. If the UN's Intergovernmental Panel on Climate Change (IPCC), is correct in its 1995 estimate that a global reforestation programme could cover 350 million hectares—an area slightly larger than the EU—then this would lead to the sequestration of up to 35 billion tonnes of carbon in 50 years. That is equivalent to soaking up around 6 per cent of projected CO_2 emissions between now and 2050.

But ideal conditions seldom exist. And apart from the scientific uncertainty, there are other problems with sequestration. Many conservationists believe that carbon credits could be disastrous for the world's surviving forests. In addition, for maximum impact most carbon-sink forests would have to be in the tropics, where trees grow fastest. But there are huge competing pressures there. The UN's Food and Agricultural Organization estimates that an extra 90 million hectares of new agricultural land will be needed, mostly in the tropics, within the next decade alone. Carbon credits would have to offer higher profits than cash crops such as rubber and palm oil.

Then there is the problem of what to do with carbon-sink forests once they have matured and are emitting, through decomposition, as much carbon dioxide as they absorb. These trees must then be removed or managed to ensure that the carbon they have locked up is not simply released again into the atmosphere.

Others claim that carbon sinks will allow industrialized nations to simply put off the more difficult task of cutting pollution at home. (Source: *New Scientist*, 24 October 1998)

Heat your greens

Sunflowers and spinach could be used to clean up spillages of radioactivity from around nuclear plants if an experiment by the British nuclear industry proves successful.

British Nuclear Fuels (BNFL) is growing dwarf sunflowers, spinach, sugar beet and Indian mustard on an

80-metre stretch of land contaminated by leaks from the Bradwell nuclear power station in Essex. The company hopes to remove radioactivity from soil by the plants' natural ability to absorb nutrients through their roots.

The ground at Bradwell was polluted in the 1970s by a leakage of liquid waste, which contained caesium 137, from an underground effluent pipe. Three years ago, after heavy rain brought some of the radioactive material to the surface, sections of the pipe and surrounding soil were dug out and disposed of at Britain's low-level waste repository at Drigg in Cumbria.

The resulting trench is still contaminated with about 100 becquerels of radioactivity per gram of soil, much higher than normal.

BNFL is considering using the technique to clean up spills at other sites. Once the plants are grown they will be incinerated, analysed for radioactivity and disposed of as nuclear waste. (Source: *New Scientist*, 1 August 1998)

Probe to catch rhino poacher

Rhinoceros horn can now be protected from poachers using DNA technology. Scientists at the National Institute of Immunology in India (NII, New Delhi) have just completed six months of tests on a DNA-detection device that has not only been shown to detect rhino horn but also to tell whether the specimen comes form India or elsewhere. Sher Ali, head of NII's molecular genetics laboratory, and his colleagues have discovered a repetitive 906 base pair DNA sequence that is unique to India's one-horned rhinoceros (*Rhinoceros unicornis*). The sequence is not present in the closely related African double-horned black rhinoceros (*Diceros bicornis*) or any other species of rhino. "This distinguishing feature will help identify if a particular horn came from India or Africa", says Ali. The great Indian one-horned rhino is an endangered species—currently numbering bout 2,000—and is confined to three or four protected forests in Assam and West Bengal in eastern India. (Source: *Nature Biotechnology*, Vol. 16, November 1998)

Increase in bio and phytoremediation market

According to the McIlvaine company in its report *Site Remediation World Markets 1998-2002*, world annual expenditures for soil and groundwater clean up using bio-remediation and phytoremediation techniques will increase from $870 million in 1997 to $1.1 billion by 2002. Despite the apparent increase in popularity of bio- and phyto-remediation, it is believed that they will account for only 5 per cent of the total $25 billion site remediation market in 2002.

Bio and phytoremediation require little capital outlay, but provide improvements more slowly than alternatives. Bio-remediation is a treatment process using naturally occurring micro-organisms to break down hazardous substances into less toxic substances and to treat contaminated soil and water. Phytoremediation is the use of plants and trees to clean up contaminants such as metals, pesticides, solvents and crude oil.

In 1997, 9.5 million cubic metres of soil were treated by bio- and phytoremediation worldwide. By 2002 the quantity is expected to rise to 12.7 million cubic metres. The largest application for bio and phytoremediation is to remediate landfills and hazardous waste dumpsites. (Source: *Engineering*, July/August 1998)

Natural cleaner

Fungi could soon become another weapon in the armoury of pollution control experts who use bacteria and plants to clean contaminated soil and water.

Bacteria break down pollutants with enzymes. But these enzymes remain within the cell, so bacteria are ineffective against chemicals that cannot enter the cell, such as those with molecules too large to pass through the cell membrane.

When white rot fungi (*Trametes versicolor*) are starved of food, however, they release enzymes outside their bodies. These enzymes are usually less specific than bacterial ones, so they could be used to tackle a wider range of pollutants. Erich Leidig of Karlsruhe University in Germany found that white rot fungus broke down 90 per cent of a dye, poly-R-4-78, commonly used to test decontamination techniques.

One reason fungi have not been used in this way before is that in an open environment they lose out to bacteria, which feed on them. Leidig, however, has found that he can protect the fungus from bacteria by encapsulating it in hydrogel—permeable to water and the fungus's enzymes, but not to bacteria.

Leidig says it would probably be most useful for cleaning waste water from the paper industry, which contains high levels of cellulose, a nutrient that is not decomposed by bacteria. (Source: *New Scientist*, 10 October 1998)

Enzyme to efficiently decompose formaldehyde

Prof. H. Yanase and his research team of the Department of Biotechnology, Faculty of Engineering, Tottori University, have discovered an enzyme that efficiently decomposes formaldehyde that is generated from the construction materials of newly built housing units.

Incorporating the enzyme in air purification systems enables formaldehyde to be decomposed and removed to create a healthy living environment. Formaldehyde is emitted form new construction materials and furniture, and is associated with sick house syndrome that causes physical illness in the occupants. The newly discovered enzyme should prevent the syndrome. The formaldehyde decomposing enzyme is called formaldehyde dismutase and was extracted from a strain of bacterium *Pseudomonas putida F61* that proliferates in soil. Formaldehyde coming into contact with the enzyme is decomposed into harmless formic acid and methanol.

Several enzymes have been discovered previously which decompose formaldehyde, but these enzymes cannot function independently, so the addition of an expensive substance as an auxiliary enzyme (coenzyme: AND) was necessary. The newly discovered enzyme requires no auxiliary enzyme and can be commercialized with ease.

The research team also succeeded in gene cloning, and clarified that the enzyme has a structure resembling that of the alcohol dehydrogenation enzyme calconel denydrogenase.

A unique characteristic of this enzyme is that it requires no auxiliary enzyme, so it can convert formaldehyde into non-toxic substances independently. In the mechanism of this reaction, AND(H) is strongly bounded with the enzyme proteins, so the auxiliary enzyme is not separated from the enzyme and performs formaldehyde oxidation and reduction simultaneously. Therefore, by fixing the formaldehyde dismutase onto filters, it will be possible to decompose formaldehyde specifically.

Formaldehyde is regarded as difficult to remove with ordinary air purification systems using activated carbon or ion

adsorbents, but the newly discovered enzyme will permit the designing of deodorizing biocatalytic films which render formaldehyde non-toxic. Further details from Tottori University, Department of Biotechnology, Faculty of Engineering, 4-101, Koyama-cho, Minami, Tottori City, Tottori Pref. 680-0945. Tel.: +81-857-31-5275; fax: +81-857-31-0881; e-mail: yanase@bio.tottori-u.ac.jp (Source: *JETRO*, June 1998)

Poplars could clear mercury from landfills

By borrowing a gene from bacteria, researchers have produced yellow poplar trees that suck mercury from the soil and convert it into a less toxic form that simply blows away. But there are worries about where this atmospheric mercury will end up.

Ionic mercury is one of the most dangerous poisons lurking in the toxic waste sites—and one of the most difficult to remove. A cheap way of eliminating it would allow large areas of abandoned land to be redeveloped. Some bacteria and plants have a natural ability to take up and detoxify metals from polluted soils, but they are small and slow-growing, and would take decades to clean a typical site.

Clayton Rugh and his colleagues at the University of Georgia, near Atlanta, have transferred a gene from mercury-resistant bacteria to a fast-growing tree, the yellow poplar. The bacterial gene, *merA*, produces an enzyme that reduces toxic mercury ions to insoluble mercury metal. Elemental mercury is volatile and evaporates from the tree leaves.

Preliminary studies of seedlings show that those with the bacterial enzyme can grow in soils with levels of mercury that are toxic to ordinary poplar seedlings. The gene increased the plants' natural ability to take up mercury by a factor of 10. Field tests are due to begin next spring.

One potential problem is that the trees can take up only soluble forms of mercury. Most contaminated sites, however, contain insoluble mercury sulphide. But mercury sulphide slowly breaks down into soluble forms—which is when it becomes dangerous, Rugh points out. "Our *merA* engineered plants would theoretically be able to remove this mobile fraction and avoid its transformation into hazardous methyl mercury", he says.

Another problem is that although the trees will remove the extremely toxic ionized mercury from the ground, elemental mercury is itself poisonous. "I would not plant these trees in a heavily populated area, for this reason", says team member Scott Merkle. "For anybody not close, the amount of mercury would be minuscule, a drop in the bucket compared to natural sources such as bacteria in soils".

There is growing international concern, however, about the long-range transport of mercury in the atmosphere. Mercury, which is emitted by fossil fuels as well as landfill sites, has turned up in the Inuit of the Canadian Arctic in concentrations above the WHO's guidelines. It apparently travels thousands of kilometres in the air before condensing out in the cold of the Arctic. (Source: *New Scientist*, 3 October 1998)

Genetically engineered bacteria to combat corrosion

Constant moisture makes the metal surfaces of pipes and tanks develop "biofilms" of bacteria that look like a black discolouration, says Barry Syrett of Electric Power Research Institute (EPRI) in Palo Alto, CA. Some kinds of bacteria reduce sulphates to sulphides that corrode metals.

Syrett and his colleagues report that aerobic bacteria such as *Pseudomonas fragi* can protect metal surfaces in the lab. The bacteria not only use up oxygen and prevent rust but also inhibit the growth of sulphate-reducing organisms, although some harmful bacteria, such as *Desulphovibrio vulgaris*, could still creep under the protective biofilm. The researchers therefore plan to genetically alter aerobic bacteria to make them release more natural antimicrobial peptides. Within a few years they hope to let engineered bacteria loose in power plants. (Source: *New Scientist*, 26 September 1998)

Industrial microbiology

Modified yeast produces more ethanol

A researcher has developed a genetically modified yeast which can efficiently ferment cellulose to ethanol—a breakthrough which could slash the costs of production of ethanol from renewable feedstocks.

The modified organism is the result of almost 20 years work by Nancy Ho, a molecular geneticist at Purdue University, West Lafayette, IN.

Amoco has licensed the yeast strain and Swan Biomass, a joint venture with Stone and Webster, is testing the yeast in a large-scale facility at the National Renewable Energy Laboratory in Golden, CO. The results have confirmed that the yeast is effective at co-fermenting glucose and xylose from cellulosic biomass into ethanol, Ho said.

Traditional brewing yeasts can only ferment the glucose component of cellulose which accounts for 60-70 per cent of the cellulose. Ho's yeast also has the ability to ferment xylose—which accounts for 30-40 per cent of cellulose. Co-fermentation of the xylose component dramatically changes the productivity of the fermentation process and also means that waste materials can be used as feedstock.

The genetically engineered yeast produces at least 30 per cent more ethanol from a given amount of plant material than the unmodified version of the yeast. It is also highly stable and does not need any special nutrients or conditions during fermentation.

"Plant material is an ideal and inexpensive feedstock for ethanol fuel production", said Ho. "This genetically engineered yeast will make it possible to substantially lower the cost of producing ethanol on a large scale. The goal is to make ethanol not only competitive with the cost of gasoline at the pump, but even much cheaper." (Source: *European Chemical News*, 21-27 September 1998)

Bean bag

Biodegradable plastic bags made form soya beans would be good for the environment. They do not take up landfill space, and because they degrade quickly they are less likely to strangle or suffocate wild animals that encounter them. There is only one problem: the plastic bags are so biodegradable that they almost dissolve in the rain.

But Joshua Otaigbe, a materials scientist at Iowa State University in Ames, has found that mixing the soya-bean protein with polyphosphate fillers and silane produces a more durable and water-resistant plastic.

In laboratory tests, the modified plastics have survived more than a year under water. The unmodified plastic dissolved in a few hours.

Otaigbe is now testing the soya-based plastics in soil to see how well they degrade. He expects the materials to be useful in a variety of roles, from food packaging to medical sutures.

The plastics will be relatively cheap, Otaigbe says, because soya beans are a renewable resource. The crop is

grown extensively in Iowa and neighbouring states in the Midwest. (Source: *New Scientist*, 21 February 1998)

Record breaking fermentation run at Yarraville

CRC researchers recently passed a major milestone in large-scale plant cell culture, with the completion of a four-month, semi-continuous fermentation programme in a 1,000 litre fermenter. Announcing the achievement, Dr. David McManus said that four months is about twice as long as the published record, and represents a significant step towards commercial operation.

By far the majority of biopolymers used industrially are derived from plants—for example starches, pectins, gum arabic and locust bean gum. The ability to produce biopolymers is shared by all plants, although the type of biopolymer produced varies from one species to another. The CRC is making the most of this natural diversity through the development of technology to produce natural gums in a controlled industrial environment, based on a technique called plant cell suspension culture.

With the right blend of nutrients and growth promoters, it is possible to encourage plant tissue to revert to a friable, amorphous state (similar to mashed potato), known as callus. When the callus is transferred to a liquid nutrient medium it breaks down further to a fine suspension of cells, which can be grown to large volumes in sterile fermentation vessels. As the cell suspensions grow, the biopolymers that normally make up the cell wall are secreted into the surrounding fluid. The product is harvested by filtering off the cells, removing unwanted salts and drying the biopolymer.

CRC researchers have found that each cell line secretes a unique blend of biopolymer components, making it possible to screen a wide range of plant species to identify gums with novel properties. To date, the CRC screening programme has examined cultures from about 150 species, and has identified several new biopolymers with interesting thickening, emulsification and gelling properties.

Promising cell lines are initially grown in shaker flasks and small fermenters (up to about 10 litres volume), to establish their basic growth and production properties, and for nutrient optimization. This work is primarily undertaken in the CRC laboratories at Food Science Australia (formerly CSIRO Division of Food Science and Technology) in Sydney.

Research then switches to the scale-up facility at Albright & Wilson's site at Yarraville, Melbourne, for further process development. This facility houses eight fermenters with volumes up to 1,000 litres, with a 10,000 litre vessel under construction. Product recovery equipment is also available, allowing production of kilogram quantities of new biopolymers for applications testing. (Source: *Australasian Biotechnology*, Vol. 8, No. 3, June 1998)

F. PATENTS AND INTELLECTUAL PROPERTY RIGHTS

Fee-free patents

The UK patent office has become the first in the industrialized world to abolish patent application fees. The move, part of a package of fee reductions, is aimed at helping small businesses and individuals protect their intellectual property.

Patent renewal fees have also been cut, by an average of 18 per cent, as have trademark application and renewal fees.

The cuts will save UK industry £12M/a, says Patent Office chief executive Paul Hartnack. "The reductions in fees make a significant contribution to the competitiveness of British industry, particularly among the small firms which are the source of innovation and creativity in Britain", he says.

The United Nations-run World Intellectual Property Organization (WIPO) is also cutting patent fees, for the second time this year. (Source: *Chemistry & Industry*, 5 October 1998)

Now you can find out who owns your genes

A database could help resolve heated debates about when sequences of DNA should or should not be patented. The idea of patenting genes has always been controversial. The debate gained impetus in the early 1990s, when some scientists started filing for patents on thousands of tiny gene fragments called expressed sequence tags (ESTs)—despite the fact that they had little idea about the function of the genes they were part of.

Last year, the US Patent and Trademark Office ruled that ESTs are patentable, amid disapproval from molecular biologists who fear that such broad-ranging patents will hinder research. But debate has been hamstrung by the lack of hard information about the contents of patents, says Stephen McCormack, associate director of the newly formed Foundation for Genetic Medicine in Manassas, VA, an independent non-profit organization.

The DNA Patents Database, compiled by McCormack and Robert Cook-Deegan of the National Academy of Sciences in Washington, D.C., now contains the full text of more than 8,500 patents. It is set up to provide the key biological information about each patent—which genes are included, the techniques used in their discovery and the precise extent of the claims made in each patent.

The database can be examined through the Foundation for Genetic Medicine's Web site at http://www.geneticmedicine.org. In the coming months, McCormack aims to broaden its scope to include patents filed in countries other than the US.

The database should be a key resource for patent lawyers and for policy analysts who want to investigate whether certain types of patents—such as those on ESTs—are hampering research. McCormack also expects funding bodies such as the US National Institutes of Health to make good use of the database. They will be able to work out exactly who is profiting from their investment in the basic research that underpins gene discovery. (Source: *New Scientist*, 28 February 1998)

Canadian patent laws to stay about the same

Canadian drug makers and biotech companies are heaving a sigh of relief—after a year-long review, draft government regulations appear to leave the patent framework essentially untouched.

While the pharmaceutical industry is pleased with the new rules, consumer groups and the generic drug makers, which had lobbied vigorously for changes, were disappointed.

At issue was a so-called "linkage" regulation that says a generic company cannot get approval to make—and stockpile—a copycat drug until the original patent has expired. The government tinkered with time limits in the regulation, but did not eliminate it.

Revisions to the Canadian Patent Act extending patent protection to 20 years were approved in 1993 after a public debate which saw economic nationalists and health-care advocates lined up with generic drug makers against the government and the pharmaceutical industry.

The revisions, known as Bill C-91, contained a provision for a review of the law four years after it was passed, a review that was completed in April and led to the new regulations. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 February 1998)

Patenting life in Europe

On 12 May 1998 the European Parliament approved the Biotechnology Patent Directive after 10 years of discussion.

One of the major rate-limiting steps of the Biotechnology Revolution has been the legal issue of prohibiting worldwide patenting of transgenic inventions. This vote removes legal prohibitions to patenting transgenics.

The following is summarized from a patent commentary by Breffni Baggot in the March 1998 issue of *Nature Biotechnology*. Two laws have blocked the patentability of transgenics: the European Patent Convention's (EPC's) Article 53(a) blocks patentability of inventions whose commercial use would be contrary to public policy and Article 53(b) excludes "plant and animal varieties" from patentability.

Interpretations vary from country to country, and therein lies the rub. The European Patent Organization (EPO) and EU member States have selectively issued or rejected patents. Two cases have made the interpretation especially difficult, the granting of a patent to the "Harvard" mouse and the rejection of a Plant Genetics Systems transgenic plant patent.

Discontent over the inability to explain this discrepancy led to the drafting of this new legislation known as the EU Biotechnology Patent Directive, which is more favourable towards patenting transgenics and less subject to idiosyncratic interpretation. The directive redefines EPC Article 53 to avoid the result in the Plant Genetics Systems case and defines both patentable plants and animals and plant and animal varieties.

If sorting out this legal process seems burdensome, similar things happened in the computer industry. The computer software industry ultimately claimed a mathematical algorithm in conjunction with a physical process to override the US Supreme Court's rejection of patenting an algorithm alone. The biotechnology industry would do well to demonstrate the industrial application of transgenic animal and plant materials.

More information can be found on the following web sites:

1. Notice of the decision, Europe OKs Biotech Patents, http://www.bric.postech.ac.kr/science/97now/98_5now/980512c.html
2. Final text of the EC Directive on the legal protection of biotechnological inventions, http://www.wuesthoff.de/c.htm
3. Commentary on the EC Directive, http://www.wuesthoff.de/e.htm, http://ci.mond.org/9506/95613.html
4. A non-technical summary of intellectual property rights issues in agricultural biotechnology, the ISB News Report Special Issue On Intellectual Property Rights, http://www.nbiap.vt.edu/news/1995/news95.may.html

Edward Wech, BioTec Innovation, Northfield, MN, eweck@microassist.com (Reproduced from ISB News Report, June 1998) (Source: *Australasian Biotechnology*, Vol. 8, No. 4, August 1998)

Sweden ratifies 1991 Act of UPOV Convention

Sweden deposited its instrument of ratification of the 1991 Act of the International Convention for the Protection of New Varieties of Plants (UPOV) on 18 December 1997, the International Union for the Protection of New Varieties of Plants (UPOV) announced.

Sweden, which was already a member State of UPOV, is the fourth State to ratify the1991 Act of the UPOV Convention (behind Denmark, Israel, and the Netherlands, all in 1996). That Act will not enter into force until one month after one additional State has deposited its instrument of adherence.

However, UPOV pointed out, Sweden has adapted its national legislation to the 1991 Act, and other States which have also done so are expected to adhere to that Act in the coming months.

The 1991 Act makes the protection of all species of plants mandatory for member States after the expiration of a transitional period and strengthens the protection afforded to plant breeders, but it leaves protected varieties available for use as a source of variation for the development of other varieties.

"The strengthened protection for plant breeders will increase the incentive provided by the Convention for plant breeding and will lessen the threat to breeders from piracy of protected plant varieties and plagiaristic breeding activities", UPOV said in its announcement.

UPOV is an acronym based on the French name Union Internationale pour la Protection des Obtentions Végétales. (Source: *World Intellectual Property Report*, vol. 12, 1998)

KIPO revamps guideline for biotech inventions

The Korean Industrial Property Office's Examination Guideline for Biotechnology Inventions is set to undergo a number of changes, under a final draft submitted recently by the KIPO Law Revision Committee (made up of KIPO officials, patent attorneys, scholars and industry researchers). KIPO expects to adopt the new Guideline as at 1 March 1998.

The revised Guideline divides biotechnology-related inventions into four groups: (a) genetic engineering; (b) micro-organisms; (c) plants; and (iv) animals. The Guideline further provides separate appendices for the preparation of "gene sequence listings in computer-readable form", "depositing and release of micro-organisms" and "description of taxonomic characteristics of micro-organisms".

Two items of special importance revised under the new Guideline are: (i) mandatory submission of a gene sequence listing in a computer-readable form; and (ii) allowance of animal patents.

Submission of gene sequence listing in computer-readable form

The new Examination Guideline essentially follows, as the standard for gene sequence listings in a computer-readable form, WIPO standard 25, to be adopted by the USPTO, EPO and JPO.

For patent applications disclosing a nucleotide sequence and/or amino acid sequence in the specification (except as prior art) filed on or after 1 March 1998, the Guideline encourages submission of gene sequence listings prepared in accordance with WIPO standard 25. The requirement will become mandatory, however, from 1999, when the electronic filing system is to be fully implemented.

A "nucleotide sequence" is defined as an unbranched linear or circular sequence consisting of 10 or more nucleotides; and an "amino acid sequence" as an unbranched linear or circular sequence consisting of four or more L-amino acids. The terms do not include nucleotides, proteins, or peptides containing D-amino acid. Amino acid sequences do, however, include sequences for a protein or peptide which contains a pseudo or non-peptide bond, cross link, or end modification.

Animal patents allowed

The revision allows animal patents, providing specific examination standards for them. The standards will apply not

only to those applications filed after the adoption of the new Guideline but also to those animal patent applications currently pending.

However, there remains the issue of what types of animal inventions may be prohibited, under Article 32 of the Patent Act, as being liable to contravene public order or morality. The new Guideline, while remaining silent on this issue, perhaps in anticipation of the full development of unsurfaced issues and the court decisions thereon, nevertheless provides the following:

Patentable subject matter

The new Guideline allows the patenting of inventions directed to an animal itself, a part of an animal, a process for creating an animal, and a use thereof. An "animal" does not include a human being.

Description requirements

(1) *Designation of animals*

An animal should be defined by its scientific name based on zoological taxonomy.

(2) *Claim drafting*

For an application claiming an animal itself, the animal should be specified by a combination of its scientific name, its characteristics, and the gene(s) conferring such characteristics. The deposit number of the embryo, or the like, of the claimed animal should be disclosed. An animal may be further specified by the process for creating it.

Example: A mouse (*Mus muscules*) having ATCC Accession No. 0000 characterized by having in its germ cells and somatic cells a gene, introduced into said mouse in the embryo step, for human beta interferon, and being able to express said human beta interferon sufficiently to provide an antiviral activity.

(3) *Detailed description of invention*

- The species of the animal created should be disclosed by the scientific name.
- Characteristics of the animal created should be disclosed by stating numeric values obtained by actual measurements, and, if desirable, may be described in comparison with those of known animals.
- Where the characteristics of the created animal cannot be expressed by a conventional breeding method, the specific breeding method and conditions should be clearly described. A process for creating the claimed animal should be disclosed to such an extent that an ordinary person skilled in the art can easily reproduce the claimed invention. In principle, this requirement is deemed satisfied when, in addition to such disclosure of the creation process, a fertilized egg or embryo capable of developing into the claimed animal is duly deposited.
- Industrial applicability, i.e. the use of the claimed animal, should be disclosed.

(4) *Drawing*

Drawings or photographs may be attached.

Patentability requirements

(1) *Novelty industrial applicability*

- Mere discovery of an animal existing in nature cannot constitute an invention having industrial applicability.

(2) *Inventive step*

- A created animal is deemed to have an inventive step if it has a characteristic which could not easily be anticipated from the known species to which it belongs.
- A created animal also has an inventive step if it provides a useful effect which could not be anticipated from the known characteristics of the animal species to which it belongs.

Sexually reproducible transgenic plant inventions remain unpatentable

Sexually reproducible transgenic plants remain unpatentable under the new standards. Article 31 of the Patent Act allows patents only for asexually reproducible plants. The issue of patent protection for sexually reproducible plants was before the Revision Committee. However, it was determined not to change their non-patentable status, in view of the possibility of their protection (along with asexually reproducible plants) under the Seed Industry Law.

The Seed Industry Law, enacted in 1996 and effective 31 December 1997, is intended to pave the way for Korea's expected membership in UPOV in 1998. While the Seed Industry Law closely follows the Patent Act in structure, the level of protection thereunder for transgenic plants follows that of UPOV.

New examination standards for genetic engineering invention

(1) *Industrial applicability*

- Inventions of a gene, vector, recombinant vector, transformant, fused cell, recombinant protein, or monoclonal antibody whose utility is not described in the specification or cannot be inferred therefrom are deemed to lack industrial applicability.
- A method of treating or diagnosing a human being is deemed to lack industrial applicability.

(2) *Novelty*

- In an invention directed to a gene, vector, recombinant vector, protein, or recombinant protein, where the claimed matter is an isolated and purified form of a single substance which is publicly known and is chemically identical to such publicly known product, the claimed matter is not novel.
- Where a recombinant process inevitably leads to a different product, e.g. in its sugar chain or the like, due to a difference in the host cells, even though the recombinant protein has the same amino acid sequence as the publicly known one, a claimed invention concerning the recombinant protein specified by the recombinant process may be novel.
- If antigen A is novel, a monoclonal antibody to antigen A is deemed novel. Where a monoclonal antibody to an epitope "a" of antigen "A" is known, a monoclonal antibody to an epitope "b" of antigen "A" will not necessarily be deemed to lack novelty.

(3) *Inventive step*

- Where protein A is publicly known but its amino acid sequence is not publicly known, an invention of a gene having a specific base sequence encoding said protein A does not have an inventive step if a person skilled in this art could determine the amino acid sequence easily at the time of filing.
- When an amino acid sequence of protein A is publicly known, an invention of a gene having a specific base sequence encoding the protein A may be ruled to have an inventive step, if such gene has a marked effect in comparison with other genes having a different base sequence encoding the protein A.

Unresolved issues of controversy

Among the issues that have seen divided opinions among the Committee members is the requirement that a priority application (whether under the Paris Convention or under the domestic priority system) must contain a micro-organism deposit number identified therein in order to claim the priority.

Further, in order to meet the enabling disclosure requirement, the Korean patent application is required to contain the deposit number or the specification cannot be amended to include the deposit number after the filing even if it can be shown that the deposit was in fact made prior to the Korean filing date. (Source: *World Intellectual Property Report*, vol. 12, 1998) (*By* C. Leon Kim, First Law Offices of Korea, Seoul; the firm may be reached at tel.: (82-2) 589-001; Fax (82-2) 589-0002; e-mail: firstlaw@users.unitel.co.kr)

International seminar adopts resolution on biodiversity

Representatives from 19 countries meeting near Bangkok on 1-6 December 1997 adopted a resolution expressing their "total and frontal opposition to the extension of intellectual property rights to life forms, be it on humans, animals, plants, micro-organisms, or their genes, cells and other parts".

Participants at the week-long seminar included representatives of indigenous, peasant, non-governmental, academic, and government organizations from Latin America, Africa and Asia.

The result of the meeting was the "Thammasat Resolution". In Thai, "Thammasat" means "knowledge of nature". As stated in the resolution, the intent of the participants was "to study, assess and develop our response to the increasing privatization of biodiversity and local knowledge, especially as driven by the Trade Related Intellectual Property Rights (TRIPs) agreement of the World Trade Organization (WTO) and the resulting legislation at the regional and national levels".

The group called for a revision of TRIPs "in order to allow countries to exclude life forms and biodiversity-related knowledge from IPR monopolies under the jurisdiction WTO". (Source: *World Intellectual Property Report*, vol. 12, 1998)

G. BOOKS, JOURNALS, REVIEWS AND BIOINFORMATICS

Biotechnology for Clean Industrial Products and Processes: Towards Industrial Sustainability (93 98 03 1 P) ISBN 92-64-16102-3

Biotechnology for Clean Industrial Products and Processes: Towards Industrial Sustainability is the report of an Ad Hoc Task Force of the OECD Working Party on Biotechnology. It was approved by the Working Party's 6th Session on 24-25 February 1998, and by the Committee for Scientific and Technological Policy on 10-11 March 1998. It continues OECD's review of environmental biotechnologies which began with *Biotechnology for a Clean Environment* (1994) and included workshops in Tokyo (1994), Amsterdam (1995) and Mexico (1996), but also opens the door to major new efforts to improve industrial sustainability in and outside OECD.

Industrial biotechnology has emerged into a world where environmental sustainability has become a global concern. The report illustrates how modern process biotechnologies can address this global concern, and how they are penetrating industrial operations in many sectors. It identifies environmental and economic advantages over other technologies, as well as technical and other bottlenecks. It also emphasizes that industry and governments must act together to respond to the challenges of industrial sustainability through biotechnology.

Environmental Impacts of Aquatic Biotechnology

This report published by OECD examines biotechnology as a means of achieving clean or cleaner industrial products and processes. It compares biotechnological processes with competing means of securing similar goals.

All stages of the life cycle of a product or process may adversely affect the environment by using up limited resources of materials and energy or by creating waste. Any substitution or change that reduces consumption of materials and energy and production of waste—including, for example, recycling of materials and energy—may be regarded as more environmentally friendly or "clean". Clean technology may also be equated with reduced risk. Life Cycle Assessment is one way of comparing the relative cleanliness of a product or process.

Chapter 1 reviews the potential role of biotechnology in clean industrial processes and sets the stage for viewing clean processes in the context of industrial sustainability. Chapter 2 discusses: (a) the main industrial sectors for which biotechnological methods appear appropriate and timely; (b) the extent to which biotechnological thinking and practice are being introduced into industrial sectors that have serious environmental impacts; and (c) the economic competitiveness of biotechnology for clean products and processes in these sectors. It gives examples of industrial biotechnology applications, organized by industrial sector, and discusses their economic impact. Chapter 3 examines scientific and technological innovations across the range of biotechnologies and the opportunities for their adoption, as well as R&D priorities. It presents the technological drivers and the additional R&D needed to introduce biotechnology for clean industrial processes and products. Chapter 4 describes life cycle concepts and the tools available or requiring development in order to make quantitative assessments of what constitutes "clean" or "cleaner" when evaluating the merits of new or alternative technologies, products or processes. Life Cycle Assessment provides a systematic means of prioritizing R&D initiatives aimed at clean industrial practices. Chapters 5 and 6 consider the role played by the general public and government in the implementation of clean technology in industry. They discuss legal and policy frameworks, public perception of biotechnology, and the need for information exchange, education and training. Chapter 7 draws conclusions and makes recommendations regarding industrial and government policies that can affect how biotechnology is used by industry to contribute to cleanliness and sustainability.

This document has been prepared by the OECD Environment Directorate, in collaboration with the Directorate for Science, Technology and Industry. It is the result of work undertaken by OECD's former Group of National Experts on Safety in Biotechnology (GNE).

A Textbook on Biotechnology by H. D. Kumar: Second Edition, ISBN 81-85938-90-3

In the second edition of this highly acclaimed introductory text, a self-contained chapter each on biobusiness, biosafety, intellectual property protection in biotechnology, and biotechnology for developing countries has been added and many new sections covering the recent developments

incorporated. Other alterations include a substantial revision of most earlier sections in all the chapters. In addition, a large number of illustrations have been introduced. Providing an overview of most aspects of general biotechnology, this updated and considerably expanded second edition, now with a length more than twice that of the previous edition, continues to serve as a basic text for any core course on biotechnology. The study would be useful also to the professional in agriculture, bioremediation, environmental health, food technology, human and animal health care industry and waste management.

Har Darshan Kumar has taught and conducted research on applied phycology and microbial and environmental biotechnology. A fellow of the three premier science academies of India and a member of several professional societies, he has since 1989 been a member of the panel on environmental effects of ozone depletion of the United Nations Environment Programme. He has also served as Coordinator of the multifaculty Biotechnology Programme in Banaras Hindu University.

Leucaena, A Genetic Resources Handbook

A new publication of particular interest to foresters and agronomists who work in the tropics is *Leucaena, A Genetic Resources Handbook*. The *Leucaena leucocephala* tree is an ubiquitous, small, seedy tree that occurs in most tropical countries. This handbook by Colin E. Hughes, with eight pages of colour plates and 68 figures, documents in 280 pages much of what was unknown about the genetic diversity within the *Leucaena* genus. It includes chapters on systematics, species characteristics, ethnobotany and indigenous domestication, hybrids, germplasm collections, seed collection and handling, conservation, and detailed information along with full botanical drawings and distribution maps. The book is freely available in English and Spanish to institutions in developing countries. In developed countries, the price is £20 plus handling and postage, with a 50 per cent discount for students. For copies or information, contact: Colin Hughes, Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB, UK. E-mail: <Colin.Hughes@plant-sciences.ox.ac.uk>. (Source: *Diversity*, vol. 14, Nos. 1 & 2, 1998)

Biodiversity and Conservation in Agriculture

The British Crop Protection Council announced publication of *Biodiversity and Conservation in Agriculture*, a compilation of papers that have been prepared by British experts who are internationally recognized in their fields. Topics range from introductory overviews of biodiversity in agriculture at habitat, species and genetic levels to field reviews of the present state of mammal, ground beetle and farmland bird populations. The effects on biodiversity when natural habitat is transformed into intensively managed arable or grassland systems are explored and a number of solutions advocated for enhancing the biodiversity of agro-ecosystems. For further information, contact Frances McKim, BCPC Editor-in-Chief, Foxhill, Stanford on Soar, Loughborough, LE12 5PZ, UK. Tel.: +44 (0) 1509 233219; Fax: +44 (0) 1509 211932; e-mail: <frances@agrisol.co.uk>or <publications@BCPC.org>. (Source: *Diversity*, Vol. 14, Nos. 1 & 2, 1998)

Measures of Success

The publication of *Measures of Success: Designing, Monitoring, and Managing Conservation and Development Projects* has been announced by Island Press and the Biodiversity Support Program (a USAID-funded consortium of the World Wildlife Fund, The Nature Conservancy and the World Resources Institute). The book is a practical, hands-on guide to designing, managing and measuring the impacts of community-oriented conservation and development projects by Richard Margoluis and Nick Salafsky with illustrations by Anna Balla. The text presents a comprehensive approach to developing and implementing conservation and development programmes, a method that has been shown to be effective by practitioners working in a broad range of projects in Latin America, Africa and Asia. The publishers say it is the only work of its kind currently available and represents a valuable resource for anyone involved in designing, monitoring and managing conservation and development projects. To obtain copies, send orders and payment to: Island Press, Box 7, Dept. 2AU, Covelo, CA 95428, USA. $35 (in the US add $5.75 for shipping. California residents add 7.25 per cent tax; Washington, D.C. residents add 5.75 per cent tax). To order by telephone, call 1-800-828-1302; outside the US and Canada, call +707-983-6432. Fax orders: +707-983-6414. Order online at <www.islandpress.org> or by e-mail to <ipwest@ igc.apc.org>. (Source: *Diversity*, vol. 14, Nos. 1 & 2, 1998)

Directory for Medicinal Plant Conservation

The *Directory for Medicinal Plant Conservation* is available on the Internet. The *Directory*, produced as part of Germany's contribution to the Clearing House Mechanism of the Convention on Biological Diversity, includes 139 medicinal plant projects and institutions, based on information from more than 80 countries, characterized by their status, objectives, activities, geographic interest, databases, publications, funding resources and contact address. The searchable database can be accessed at <http:\\www.dainet.de/genres/mpc-dir>. Further information is available from Dr. Uwe Schippmann, Bundesamt für Naturschutz, Konstantinstrasse 110D, 53179 Bonn, Germany. Fax: (49) 228 9543470; e-mail: <schippmu@bfn.de>. (Source: *Diversity*, vol. 14, Nos. 1 & 2, 1998)

Biotechnology Patents in the Pharmaceutical Industry

Biotechnology inventions are becoming increasingly important in the health-care arena but protecting them with patents is not as straightforward as it is with traditional drugs. The recent resurrection of the European Biotechnology Patenting Directive has once again raised the issues surrounding biotechnology patenting, including the moral and ethical problems it presents in the minds of the public.

Biotechnology Patents in the Pharmaceutical Industry by Beverly Swain and published by the *Financial Times Healthcare* addresses why patents are so vital for the research-based pharmaceutical industry and explores all of the key issues surrounding biotechnology patents. The book reviews all aspects of biotechnology patenting within the most important markets of the US, Japan and Europe.

This report reviews the leading companies/institutions in terms of the number of patent applications filed, as well as the patent portfolios of the most commercially successful biotechnology companies. The often complex patent position of some leading biotechnology products is also classified.

Biotechnology products have also given rise to much litigation as the issues of ownership are fought out in the

patent courts. This report looks at some notable biotechnology cases, examining the issues of novelty of the inventions, obviousness and sufficiency. The outcomes of these cases, including out-of-court settlements and cross- licensing deals, are reviewed.

The report includes the number of patent applications filed compared to the number granted, the country filing pattern of biotechnology inventions and geographic analysis of where biotechnology inventions are being made. Therapy areas claimed and new subject areas being patented are also detailed.

Complex patenting matters are discussed in a format accessible to non-patent and patent experts alike. This report explains in clear and concise terms what is legally patentable, what is actually being patented and how these patents are performing in the market place.

ISBN 1-86-067-359-7: *Biotechnology Patents in the Pharmaceutical Industry*, price £525/US$ 830. Discounts available: 10 per cent when ordering 2 reports, 15 per cent for 3, 20 per cent for 4+. Please note: these charges exclude any local taxes and import duties.

Available from: Marketing Department, FT Healthcare, Maple House, 149 Tottenham Court Road, London W1P 9LL, UK. Tel.: +44 (0) 171 896 2066; Fax: +44 (0) 171 896 2449; Internet: www.fthealthcare.com.

Biotechnologies in developing countries

Albert Sasson's *Biotechnologies in developing countries: present and future* is such a wide overview that it has been published in two heavy volumes over a five-year period. Largely a biotech believer, Sasson has put together a wealth of information on how different actors are shaping the introduction of modern biotechnologies in developing countries.

Volume 1: Regional and national survey (1993) reviews individual countries' economies and policies, institutional frameworks, and even includes the names of the main involved companies. Less developed countries are also covered, although very briefly if they do not hold large biotech R&D programmes. The volume is a very good picture of what countries were working on in the early 1990s.

Volume 2: International co-operation (1998) overviews the role of the United Nations agencies, the CGIAR, the EU and others in promoting modern technologies in developing countries. It also provides good information on the activities of private companies. Short subsections, boxes and an index would have made the huge amount of information contained in the book more easily accessible, but it is still a valuable resource.

Albert Sasson, *Biotechnologies in developing countries: present and future. Volume 1: Regional and national survey* (1993), 764 pp., ISBN: 93-3-102875-8, and *Volume 2: International co-operation* (1998), 806 pp., ISBN: 92-3-103460-X. Each volume is priced at FF 280, both for FF 500. UNESCO Publishing, 1, rue Miollis, 75732 Paris Cedex 15, France. Fax: (33-1) 45 68 57 41. Internet: http://www.unesco.org/publishing. (Source: *Seedling*, September 1998)

An Introduction to the WTO Agreements and The WTO Agreements Deficiencies, Imbalances and Required Changes

The Third World Network has just published two companion books, *An Introduction to the WTO Agreements* and *The WTO Agreements Deficiencies, Imbalances and Required Changes*, written by India's Ambassador and Permanent Representative to GATT, Bhagirath Lal Das. The first book is a simple, concise, non-technical exposition of the meaning or the contents of the various WTO agreements, which includes only some introduction to the issues. The book may be useful to anybody (from NGO activists to government officials to business executives) who needs to learn the basics of the new multilateral framework of international trade. Value judgements have been left for the second of the companion books, where the author proposes developing countries reflect on why they gave away far more concessions than they received, and on the ways the current agreements should be changed. The author's starting point is an acceptance of WTO as an international forum for negotiation, which may be unacceptable to some people following the debates. However, this may be its strongest asset, since the book carries a strong critique of the system and proposals for real change in the language delegates in these forums do understand.

Bhagirath Lal Das, *An Introduction to the WTO Agreements* (1998), 138 pp., ISBN: 983-9747-27-4 and *The WTO Agreements Deficiencies, Imbalances and Required Changes* (1998), 122 pp., ISBN: 983-9747-25-8. Part of the series: Trade and Development Issues and the World Trade Organization. Third World Network, 228 Macalister Road, 10400 Penang, Malaysia. Fax: (60-4) 226 45 05. (Source: *Seedling*, September 1998)

Strengthening the scientific basis of in situ conservation of agricultural biodiversity on-farm

IPGRI's latest publication on the scientific basis of *in situ* conservation is based on the premise that the *in situ* conservation of crop plants involves the on-farm conservation of local crop cultivars (or landraces) with the active participation of farmers. This clear and well-presented document summarizes the outcome of a meeting in which scientists, NGO and donor representatives identified what data needs to be collected to link farmer decision-making to genetic diversity and which methodologies are available to add value to local cultivars. This exploration of options for data collecting and analysis is linked to IPGRI's global programme to strengthen the scientific basis of *in situ* conservation of agricultural biodiversity in nine countries: Burkina Faso, Ethiopia, Nepal, Viet Nam, Peru, Mexico, Morocco, Turkey and Hungary. In each country, the strengthening of relations between formal sector institutions and both farmers and local level institutions to promote on-farm conservation is a major objective.

Debra I. Jarvis and Toby Hodgkin, *Strengthening the scientific basis of* in situ *conservation of agricultural biodiversity on-farm. Options for data collecting and analysis*, International Plant Genetic Resources Institute, 1998. 104 pp., ISBN 92-9043-370-1. IPGRI, Via delle Sette Chiese 142, 00145 Rome, Italy. (Source: *Seedling*, September 1998)

Experimenting within farmers' worldviews

Experimenting within farmers' worldviews is the outcome of the inception workshop of an innovative and courageous learning and action-oriented initiative known as COMPAS. COMPAS is about enhancing endogenous development by building on indigenous knowledge and practices, through cooperation with indigenous institutions and by understanding, testing and improving on existing indigenous practices. The document reads like research planned and carried out within an alternative paradigm: the need to

combine creativity with rationality, dealing with the issues of power and property rights, integrating gender perspectives and generation issues, learning with rural people, conducting local experiments within the farmer's cosmovision, farmer to farmer exchange, networking and advocacy. As such, the contributors to this document break new ground and have laid the basis of an important initiative.

ETC-COMPAS, *Experimenting within farmers' worldviews. Report of the inception workshop*, COMPAS, February 1998. Available from ETC-COMPAS, P.O. Box 64, 3830, AB Leusden, The Netherlands. Fax: (31-33) 49440791; E-mail: compas@etcnl.nl. (Source: *Seedling*, September 1998)

UNEP publications

UNEP has released some more in its "Environment and Trade" Series: Volume No. 9 (1995), *Dispute Avoidance and Dispute Resolution in International Environmental Agreements and Multilateral Trade Agreements: An Introduction*, 74 pp.; No. 11 (1995), *Economic Policy Reforms and the Environment: African Experiences*, 214 pp.; No. 12 (1995), *The Use of Economic Instruments in Carbon Dioxide Mitigation: A Developing Country Perspective*, 99 pp. All three are ISSN 1020-1610; No. 13 (1997), *Criteria in Environmental Labelling. A Comparative Analysis of Environmental Criteria in Selected Labelling Schemes*, 148 pp.; No. 14 (1997), *Trade-related Environmental Measures in the Field of Safety in Biotechnology*, 51 pp. Both are ISSN 1020-20.

Available from: UNEP, 15, chemin des Anemones, CH-1219 Chatelaine, Geneva, Switzerland. Fax: (41-22) 796 9240. Series Coordinator: Scott Vaughan. (Source: *Seedling*, September 1998)

IPGRI publications

IPGRI has released a new volume in its International Crop Network Series: No. 12 (1998), *International Beta Genetic Resources Network*, 104 pp., ISBN 92-9043-368-X). Also from IPGRI, in relation to the ECP/GR, are the proceedings of an international conference on crop germplasm conservation: *Challenges in rye germplasm conservation* (1996, 120 pp., ISBN 92-9043-371-X). A second volume related to ECP/GR is: *Report of a Working Group on Malus/Pyrus* (1998, 106 pp., ISBN 92-9043-376-0). Also available is the *Report of a Working Group on Forages* (1998, 194 pp., ISBN 92-9043-379-5), the proceedings of a meeting held in March 1997 in Norway.

All these publications are available from: IPGRI, Via delle Sette Chiese 142, 00145 Rome, Italy. (Source: *Seedling*, September 1998)

BUKO publications

BUKO has released the proceedings of *In Safe Hands*, the NGO and PO preparatory meeting to the 4th International Conference on Plant Genetic Resources, which took place in Leipzig, Germany, in June 1996. Although a little late, it is still a very useful document. It outlines strategies for food security, land reform, cultural self-determination and consumers' and farmers' rights, which were developed by more than 120 representatives from both North and South present at the meeting. This publication includes the major NGO conference documents, and includes English, Spanish and French versions of the *Leipzig Commitment to Agricultural Biodiversity: Towards a People's Plan of Action.*

BUKO Agro Coordination, *In Safe Hands*, Forum Umwelt & Entwicklung, Bonn, 1998, 68 pp. For copies contact: Forum Umwelt & Entwicklung, Am Michaelson 8-10, D-53177 Bonn, Germany. Fax: (49-228) 35 90 96; e-mail: <forum.ue@t-online.de>. (Source: *Seedling*, September 1998)

Biological and Cultural Diversity

Biological and Cultural Diversity: Challenges and Proposals from Latin America is a special issue of *Beyond Law*, a journal on law and social change published in Bogotá. It brings together a collection of writings and legal analysis by the members of the Colombian Ad Hoc Group on Biological Diversity, an interdisciplinary team that in recent years has played an important role in shaping the national and regional debates around IPRs and frameworks for collective/ community rights. The articles are divided into five thematic areas: collective rights, international negotiations, strategies for use and conservation of biological diversity, cultural and biological diversity, and recent legal developments in Colombia.

Grupo Ad Hoc, *Biological and Cultural Diversity: Challenges and Proposals from Latin America*, Issue 18-19, *Beyond Law*, ILSA, Bogotá, 1998, 270 pp. For copies contact: ILSA, Calle 38 No. 1645, Bogotá, Colombia. Fax: (57-1) 288 48 54; e-mail: silsa@col1.telecom.com.co; http://www.ilsa.org.co (Source: *Seedling*, September 1998)

An Appraisal of the Working in Practice of Directive 90/220 concerning the Deliberate Release of Genetically Modified Organisms into the Environment

This report, by Rene von Schomberg, makes an analysis of the actual working in practice of this directive and reviews the European Commission's proposal to amend this directive by the European Parliament and the Council during 1998. The report evaluates the Commission's proposal (among others: fixed-term licence for market products/monitoring/ introduction of risk categories/administrative procedures) and the concerns of environmental and industrial organizations. The report concludes that we should move away from a risk-focused regulatory framework to a social- embedded uncertainty-based policy. An appropriate understanding of the precautionary principle would stimulate deliberation-based policies in Member States in order to meet both environmental standards and industrial and social needs. The report makes recommendations on how to facilitate such a new regulatory framework.

To order a free copy of the report, send a fax to: STOA European Parliament, Bâtiment Schuman 4/82, L 2929 Luxembourg. Fax: (International)—352-4300 22418; e-mail: Rholdsworth@europarl.eu.int

New Fertilizer Manual released

IFDC and the United Nations Industrial Development Organization (UNIDO) recently released the third edition of the *Fertilizer Manual*, which was published by Kluwer Academic Publishers.

This new, fully updated, comprehensive reference on the technology of fertilizer production contains 22 chapters on fertilizer use, production and distribution economics, raw materials and the status of the fertilizer industry with demand-supply projections. Also included are engineering flow diagrams and process requirements for the primary fertilizer processes, including ammonia, urea, phosphates, potassium products and many others. Environmental considerations are addressed clearly. Professionals involved in any phase of fertilizer production, marketing, distribution, or use will find this book valuable.

The publication of this edition of the *Fertilizer Manual* is especially timely in that the World Food Summit, which was held in 1996, discussed the global challenges created by a burgeoning population, shrinking land area available for food production, and mounting food insecurity. The United Nations forecasts that agricultural output must be tripled if the 8.5 billion population of the world of 2025 is to be fed; with increasingly limited land under cultivation, sustainable food security cannot be achieved without the benefits of intensified agriculture—the key to alleviating poverty.

The last revision of the *Fertilizer Manual* was published in 1979. Since that time outstanding advances in fertilizer technology have occurred, whereby more energy-efficient processes and reductions in the cost of production have resulted. This edition of the *Fertilizer Manual* provides planners with information on these new advances.

Interested parties may obtain a copy of the *Fertilizer Manual* (IFDC-R-11) by placing an order with the IFDC Purchasing Department, P.O. Box 2040, Muscle Shoals, AL 35662, USA. E-mail: purchasing @ifdc.org; or Fax: 256-381-7408. The price of the publication is US$ 60 for US addresses and US$ 75 for non-US addresses. The price includes shipping and handling. (Source: *IFDC Report*, June 1998)

Traditional Foods: Processing for Profit

This is a wide-ranging guide to the processing of traditional food from many countries in Asia, Africa and Latin America, providing technical information for small food businesses. Part one deals with the basic concepts of hygiene, processing methods and quality assurance. Part two covers all kinds of processed food products, with particular emphasis on quality control aspects of raw material selection, processing and packaging. This book is relevant to development workers involved in supporting small enterprise development programmes, aid agencies, and students of food science or similar disciplines.

Peter Fellows (ed.), *Traditional Foods: Processing for Profit*, Intermediate Technology Publications Ltd., 1997, 288 pp., ISBN 1-85339-228-6. Available at £25.00 or $47.50 from Intermediate Technology Publications Ltd., 103-105 Southampton Row, London WC1B 4HH, UK. Fax: (44-171) 436 20 13. (Source: *Seedling*, December 1997)

Integrated Pest Management in Asia and the Pacific (1996)

The Asian Productivity Organisation has just published two books tackling issues on integrated pest management and technology management in Asia and the Pacific. *Integrated Pest Management in Asia and the Pacific* (1996) gives an overview of the current status of development, specifically of government-initiated integrated pest management programmes in Asia and the Pacific. *Prospective Agricultural Technologies in Asia and the Pacific* is a more recent publication (1997), presenting the status of agricultural technologies in the region as well as the prospects for development of new agricultural technologies such as biotechnology, computerization and mechanization, etc.

Free for Asian non-governmental organizations, contact: Asian Productivity Organisation (APO), Aoyama Dai-ichi mansions, 4-14, Akasaka 8-chome, Minato-ku, Tokyo, Japan. Fax: (81-3) 3408 7220; e-mail: apo@gol.com, or visit their Web page at http://www.ftf-tokyo.com/apo-tokyo/ (Source: *Seedling*, December 1997)

Human Nature: Agricultural Biodiversity and Farm-Based Food Security

Human Nature: Agricultural Biodiversity and Farm-Based Food Security is an independent study prepared by RAFI for the Food and Agriculture Organization of the United Nations (FAO). The book provides an introduction to agricultural biodiversity, with chapters assessing the current situation for each major sector: crop genetic resources, farm animal diversity, fish and aquatic life, forests, soil biodiversity and microbial genetic resources. It concludes with a discussion of outstanding policy issues that must be addressed by policy makers and global civil society. The book argues strongly that it is impossible to talk about the conservation and sustainable use of genes, species and ecosystems separately from human cultures. Farming and indigenous communities hold the key to conservation and use of agricultural biodiversity, and to food security for millions of the world's poor. Richly illustrated with graphs, charts and photographs.

Hope Shand, *Human Nature: Agricultural Biodiversity and Farm-Based Food Security*, RAFI, Canada, 1997, 94 pp., ISBN 0-9683112-0-2. US$ 20 by airmail, US$ 10 by surface. Southern NGOs receive a 50 per cent discount. Inquiries to: RAFI, suite 504, 71 Bank St., K1P 5N2 Ottawa, Ontario, Canada. Fax: (1-613) 567 68 84; e-mail: news@rafi.org (Source: *Seedling*, December 1997)

New Seed and Old Laws: Regulatory reform and the diversification of national seed systems

This book concludes that local level seed access and management is increasingly important to strengthen local, sustainable and self-sufficient farming systems. As governments—often under monetary structural adjustment pressures—have withdrawn from agricultural support, transnational corporations have aggressively moved in to market their standardized crops and implant northern-style IPR laws. Yet, the book argues, there is considerable space and justification for national seed systems that are responsive to local smaller farmers. The book is the result of a three-year project by the Overseas Development Institute (ODI) on seed regulatory reform, and includes case studies from Bolivia, India, Kenya, Nepal, Pakistan, the Philippines and Zimbabwe.

Robert Tripp (ed.), *New Seed and Old Laws: Regulatory reform and the diversification of national seed systems*, Intermediate Technology, London, 1997, 259 pp., ISBN 1-85339-415-7. At £12.95, the book is available from: IT Publications Ltd., 103-105 Southampton Row, London WC18 4HH, UK. Fax: (44-171) 436 20 13. (Source: *Seedling*, December 1997)

Seeds and Survival: crop genetic resources in war and reconstruction

This book addresses a crucial and understudied problem. Most of the current so-called low-intensity conflicts (LICs) flare up in poorer tropical and subtropical countries. At the same time, there is evidence that the usual massive dumping of foreign, non-traditional foods tends to hamper longer-term food security recovery and may encourage genetic erosion. The book presents three country case studies (Liberia, Sierra Leone and Guinea-Bissau) on the effects of war for farmer-based systems in the West African rice zone. Recovery for rural communities may mean more than just social aspects, facilitating new alliances where agrarian relationships have been disrupted, and flexibility on the part of technicians managing agricultural support. Where seeds have been lost,

both the re-establishment of informal exchange and well-designed germplasm flows from *ex situ* sources are important.

P. Richards and G. Ruivenkamp, *Seeds and Survival: crop genetic resources in war and reconstruction*, IPGRI, Rome, 1997, ISBN 92-9043-349-3, 65 pp. Request copies from: IPGRI, Via delle Sette Chiese 147, 00145 Rome, Italy. (Source: *Seedling*, March 1998)

Medicinal Plants and Herbal Medicine in Africa: Policy Issues on Ownership, Access and Conservation

This is the report of the first Organisation of African Unity-sponsored workshop on the subject, held from 14 to 17 April in Nairobi. Faced with increasing development and commercialization pressure, African countries urgently need to assess their medicinal plant genetic resources, and it is recommended that traditional practitioners be tapped as para-taxonomists. The report comes through strongly against unrestricted bioprospecting and warns against continued appropriation by developed countries of local resources and knowledge. The workshop delivered interesting recommendations on, among other things, community-sustainable use, farmers' rights linked to land tenure, differentiated access rights for local communities and traditional healers—even within reserves and natural areas—and the development of community rights regimes distinct from Western-style IPRs.

OAU/STRC/DEPA/KIPO, *Medicinal Plants and Herbal Medicine in Africa: Policy Issues on Ownership, Access and Conservation*, BDCP Press, Nairobi, 1997, 32 pp. Available from: BDCP, 110 Aku Road, Nsukka, Nigeria. (Source: *Seedling*, March 1998)

Farming systems approaches for the sustainable use and conservation of agricultural biodiversity and agro-ecosystems

A meeting on this subject, jointly organized by FAO and the CBD with the support of the Netherlands, was held in Rome in June 1997. The participants met to consider how to address agricultural biodiversity in an integrated manner. On the table were decisions by CBD, the declaration of the World Food Summit, recommendations by FAO's Commission on Genetic Resources for Food and Agriculture, and the Leipzig Global Plan of Action. The recommendations deal with increased information and awareness, support to countries for implementing systems approaches to agricultural biodiversity, and coordination among international bodies.

Patrick Mulvaney (editor), *Farming systems approaches for the sustainable use and conservation of agricultural biodiversity and agro-ecosystems*, FAO, 1997, 57 pp. Request copies from: Commission on Genetic Resources for Food and Agriculture, FAO, Via delle Terme di Caracalla, 00145 Rome, Italy. (Source: *Seedling*, March 1998)

Animal-To-Human Organ Transplants: A Medical And Legal Perspective

These are the proceedings of the fourth International Scientific Congress organized by anti-vivisection group Doctors and Lawyers for Responsible Medicine (DLRM). The articles provide concise but interesting information on the history, the actors and the dangers of xenotransplantation.

Fourth International Scientific Congress, Vancouver, July 1997. *Animal-To-Human Organ Transplants: A Medical And Legal Perspective*, Doctors and Lawyers for Responsible Medicine, 1997, 28 pp., London. Available from: DLRM, 104b Weston Park, London, N8 9PP. Fax: (44 181) 342 98 78. (Source: *Seedling*, March 1998)

Understanding Biodiversity: an agenda for research into biodiversity

This report, which was prepared by the European Working Group on Research and Biodiversity (EWGRB), was commissioned by the European Commission as the guideline for the necessary research that will allow Europe to fulfil its commitments under the Convention on Biological Diversity. The agenda includes—and not merely in a superficial way—biodiversity within farming systems. The report insists on the need to have a multidisciplinary, multi-actor approach to biodiversity use, evaluation and conservation. The fact that it will set the guidelines for the funding of projects on biodiversity research makes it interesting for organic farmers, environmental organizations and scientists.

EWGRB, *Understanding Biodiversity: an agenda for research into biodiversity*, Directorate-General XII for Science, Research and Development, Commission of the European Communities, Stockholm and Brussels, October 1997. Available from: EC DG-XII/D/1, 8, Square de Meeus, B-1040 Brussels, Belgium. Fax: (32 2) 295 20 97. Internet: http://www.europa.eu.int/en/comm/dg12/envir.html (Source: *Seedling*, March 1998)

Coastal Area Management in South Asia: A Comparative Perspective

In September 1996, the International Collective in Support of Fishworkers (ICSF) organized the South Asia Workshop and Symposium on Fisheries and Coastal Area Management. The Conference, which was attended by delegates from India, Bangladesh, Sri Lanka and the Maldives, dealt with the main issues affecting coastal area management, such as common property and property rights, fisheries management in the context of integrated coastal area management and shrimp aquaculture, and the international legal instruments available to implement community-controlled coastal area management. The workshop discussions were partly fuelled by the preparatory work by Chandrika Sharma of ICSF, who prepared the now also published background paper *Coastal Area Management in South Asia: A Comparative Perspective*, which includes overviews for Sri Lanka, India, Bangladesh and the Maldives.

Proceedings, South Asia Workshop and Symposium on Fisheries and Coastal Area Management, 26 September-1 October 1996, Madras, India. ICFW, 1997, 143 pp. Chandrika Sharma, *Coastal Area Management in South Asia: A Comparative Perspective*, Background Paper, 1997, 34 pp. Both are available from: ICSF, 27 College Road, Chennai, 600 006, India. Fax: (91 44) 825 44 57. (Source: *Seedling*, March 1998)

The Parts of Life: Agricultural Biodiversity, Indigenous Knowledge, and the Role of the Third System

This is the third part of a trilogy by Pat Mooney on the struggle by Civil Society Organisations (CSOs) for control over the biological resources upon which we all depend for our food and other livelihood needs. Written in Mooney's sharp and witty style, the book takes us through 20 years of political developments in the arena of genetic resources, and then gives us a detailed analysis of the international events concerning agricultural biodiversity in 1996. The book also critically examines the Green Revolution and international

agricultural research, corporate control over biological diversity, and the patenting of life. In the final section Mooney takes a searing look at the history and record of CSOs during the past decades, and concludes that even if there have been no great victories, today more than ever civil society—faced with TNC might and governmental meekness—must take the lead against top-down globalization.

Pat Roy Mooney, *The Parts of Life: Agricultural Biodiversity, Indigenous Knowledge, and the Role of the Third System*, Development Dialogue, special issue, Dag Hammarskjöld Foundation, Uppsala, April 1998, 184 pp., ISSN 0345-2328. For copies contact: Dag Hammarskjöld Centre, Övre Slottsgatan 2, SE-753 10 Uppsala, Sweden. (Source: *Seedling*, June 1998)

The Diversity and Dynamics of Shifting Cultivation: Myths, Realities, and Policy Implications

This is a welcome contribution which should help correct misconceptions which lead to farmers in the Third World tropics being blamed for forest destruction. Contrasting commonly held misconceptions with reality, shifting cultivation is demonstrated to be agro-ecologically sound, encompassing a remarkably diverse range of practices and ecological settings. It is often highly productive and integrated into wider livelihood systems, resulting in sustainable management and enhanced biodiversity. Development agencies, agricultural researchers and policy makers should take heed, and rethink their prejudices at this type of agriculture which has sustained—and still feeds—hundreds of millions.

Lori Ann Thrupp, Susanna B. Hecht and John O. Browder, *The Diversity and Dynamics of Shifting Cultivation: Myths, Realities, and Policy Implications*, World Resources Institute, Washington, D.C., 1998, 48 pp., ISSN 1-56973-230-2. For copies: Center for Sustainable International Development, WRI, 1709 New York Ave., NW, Washington, D.C., 20006, USA. Internet: http://www.wri.org/wri (Source: *Seedling*, June 1998)

LifeStrains Biotechnologies Resource Guide

The LifeStrains Project is an initiative of the Vancouver-based Pomelo Project, a collective engaging in contemporary cultural politics. The increasing concerns raised by biotechnology has led the LifeStrains Project to publish a user-friendly, inspiring *LifeStrains Biotechnologies Resource Guide* of resources conceived "to help organise a strong resistance to developments in biotechnologies which compromise the quality of our lives". The Guide contains a directory of organizations, a list of publications, a selection of Internet resources, a (very well) annotated bibliography and a bibliography. Although the directory is not exhaustive, it does cover many issues and, through its annotated bibliographies, provides critical insights.

Marnie Thorp, *LifeStrains Biotechnologies Resource Guide: directory, Bibliography, annotated Bibliography*, Pomelo Project, 1997, 82 pp. Available from: The Pomelo Project, P.O. Box 122, 125A-1030 Denman Street, Vancouver, BC, Canada V6G 2M6. E-mail: thorp@sfu.ca (Source: *Seedling*, June 1998)

Seeds of Change

Oxfam Belgium has started a new publication series for its Food Sovereignty Campaign, *Seeds of Change*. The series will be produced in French, English and Spanish. The first issue is devoted to the implications of patents on seeds and biopiracy is a great introductory document.

"The colonisation of life itself", *Seeds of Change, Food Sovereignty Campaign Bulletin*, Oxfam Solidarity Belgium, Brussels. Available from: OXFAM Solidarity Belgium, rue du Conseil 39, 1050 Brussels. Tel.: (32-2) 512 99 90; Fax: (32-2) 511 89 19; e-mail: isabelle.delforge@ oxfamsol.ngonet.be (Source: *Seedling*, June 1998)

Seeds of Suicide: The ecological and human costs of globalisation of agriculture

This booklet addresses the real causes of last year's Indian cotton farmer suicides in Andhra Pradesh. Increased dependency on cash crops, high external input expenses, and vertical integration of pesticide and credit schemes leave farmers very vulnerable to climatic stress and pest outbreaks. The booklet anticipates that the introduction of transgenic *Bt* cotton will only exacerbate things. *Betting on Biodiversity: why genetic engineering will not feed the hungry* skilfully argues how the new gene technologies will create new biodiversity losses, increase weed and pest problems, and further displace Third World farmers. It includes a searing critique of "bad science"—as put forward by corporate scientists.

Seeds of Suicide: The ecological and human costs of globalisation of agriculture (Vandana Shiva and Afsar H. Jafri, RFSTE, 1998, 40 pp.), *Betting on Biodiversity: why genetic engineering will not feed the hungry* (Vandana Shiva, RFSTE, 1998, 57 pp.). Both available from: Research Foundation for Science, Technology and Ecology/RFSTE, A-60 Haus Khas, New Delhi, 110 016, India. Fax: (91-11) 685 67 95; e-mail: vshiva@giasd101.vsnl-net.in (Source: *Seedling*, June 1998)

Eat Your Genes: how genetically modified food is entering our diet

A full-fledged overview of genetic engineering in agriculture. It starts introducing the technology, to follow on with the main research and development lines the industry is engaged in, the main ecological risks, patenting, biosafety regulations, labelling, the impacts of biotechnology in the Third World. Although the book does not contain new criticisms of genetic engineering, nor critiques the reductionism underlying this technology, it does provide a wealth of information that give it the potential to be a most useful resource tool.

Stephen Nottingham, *Eat Your Genes: how genetically modified food is entering our diet*, Zed Books, London, 1998, 212 pp., ISBN 1-85649-578-7. Order from: Zed Books Ltd, 7 Cynthia Street, London N1 9JF, UK. Priced at US$ 17.95 paperback and US$ 45 hardback. (Source: *Seedling*, June 1998)

Strategic Decisions for Agricultural Biotechnology: Synthesis of Four Policy Seminars

This is an *ISNAR Briefing Paper* which analyses the main constraints countries report as preventing the development of biotechnology in the South, and comes up with a strategy to overcome these by: developing a policy on an agenda for biotechnology research; forging partnerships; and providing incentives for the private sector. It is interesting to see where mainstream agricultural research is heading.

Joel I. Cohen et al., "Strategic Decisions for Agricultural Biotechnology: Synthesis of Four Policy Seminars", *ISNAR Briefing Paper*, No. 38, ISNAR, The Hague, 1998, 11 pp., ISSN 1021-2310. Available from ISNAR, Laan van Nieuw Oost Indië 133, 2593 BM The Hague, P.O. Box 93375, 2509 AJ The Hague, The Netherlands. Tel.: (31-70) 349 61 00; fax:

(31-70) 381 96 77; e-mail: isnar@cgnet.com (Source: *Seedling*, June 1998)

Proprietary Biotechnology Inputs and International Agricultural Research

This is another *ISNAR Briefing Paper* which presents the results of a task force to develop the CGIAR policy on IPRs. The study recommends that the CGIAR learns how to work in an IPR environment, coordinates its policies regarding IPRs and access to proprietary technologies and materials, and reviews the guidelines for managing intellectual property at individual centres. It also considers possibilities for a system-wide policy.

Joel I. Cohen et al., "Proprietary Biotechnology Inputs and International Agricultural Research", *ISNAR Briefing Paper*, No. 39, ISNAR, The Hague, 1998, 11 pp., ISSN 1021-2310. Available from ISNAR, Laan van Nieuw Oost Indië 133, 2593 BM The Hague, P.O. Box 93375, 2509 AJ The Hague, The Netherlands. Tel.: (31-70) 349 61 00; fax: (31-70) 381 96 77; e-mail: isnar@cgnet.com (Source: *Seedling*, June 1998)

New on-line magazine tackles biotechnology issues

The Illinois-Missouri Agricultural Biotechnology Alliance (IMBA) is pleased to announce *AgBioForum*, a quarterly on-line-magazine which publishes short, timely articles on the economics and management of agricultural biotechnology. Contributors are academics, private and public sector analysts and decision makers. *AgBioForum* can be found at www.agbioforum.missouri.edu and is available free of charge. *AgBioForum* is intended for a wide audience, including academia, industry, government, agribusiness media and students.

The first issue of *AgBioForum* focuses on the public acceptance of agricultural biotechnology, a topic that produced front page material long before any commercial product was in sight. There have been several concerns about agricultural biotechnology, including perceived environmental and food safety risks and the potential for ethical and religious conflicts. The summer 1998 issue of *AgBioForum* provides viewpoints of academic and industry experts from the US, Europe and Canada. (Source: *News Release*, 31 August 1998)

Genes on the Internet

The UK Agricultural Biodiversity Coalition—a project of the UK Food Group—has set up a new, ambitious Web page covering agricultural biodiversity issues. Rather than producing new information, the Web site is a welcome effort to put together the work of the many NGOs involved in issues related to the conservation and sustainable use of agricultural biodiversity—including livestock and fisheries. The visitor is invited to read selected, "fished" materials, or to go to the Web pages of the many more specialized NGOs. Special emphasis is given to the conservation and use of genetic diversity in Europe.
http://ds.dial.pipex.com/ukfg/ukabc.htm

Great news for those of us following patents on life. Finally, the European Patent Office is giving free Internet access to the bibliographic data and the text of European patents. In an effort shared by all national patent offices, esp@cenet has been put into place. Esp@cenet allows searches on all EP and EPC member States, WIPO, US and Japanese patents. Full patent information is retrievable for those European and WIPO patents published during the last two years, and abstracts are available from 1970 onwards.
http://ep.dips.org/dips/ep/en/dips.htm

And if in your patent research you have problems in coming to terms with the international patent classification codes, and you understand French, your best option is to go to the French patent office Web page and find out what each code means: http://www.inpi.fr/inpi/html/cibaide.htm

Japanese patents can be searched (in English!) at http://www2.jpo-miti.go.jp/ (Source: *Seedling*, September 1998)

The Research Foundation for Science, Technology and Ecology (RFSTE) has recently set up a Web page. RFSTE has managed to put together the main issues it works on in a clear, powerful, easy-to-read format. The Web page offers information at different levels of difficulty, from the very basic (and quite funny "Simple Discussion of Biodiversity: Everything you always wanted to know about biodiversity but were afraid to ask"), to a far more complex presentation of the issues around biopiracy, biotechnology, globalization and food security, among others. Although the issues dealt with have international connotations, the Web page has a welcome Indian slant. RFSTE's main activities and publications are also posted.
http://www.indiaserver.com/betas/vshiva/

The Physicians and Scientists Against Genetically Engineered Food have set up a Web page on genetically engineered food safety problems. At a time when campaigners against genetic engineering in agriculture are so often accused of being "anti-science", we welcome this group of committed scientists who strongly critique the technology from a "scientific point of view". The Web page also contains a Declaration outlining the group's principles, which is open for new signatures. Not to be missed.
http://home1.swipnet.se/~w-18472/indexeng.htm

The Genetics Forum, a UK-based NGO with a long involvement in the fight against gene technology and patents on life, has also set up a new Web page, where it introduces itself and lists some of the most interesting articles from its magazine, *The Splice of Life*. The page also contains a calendar of international activities in the arena of biodiversity and genetic engineering. It is still under construction, and if you would like to have links with their Web page, go visit!
http://www.geneticsforum.org.uk/

The Friends of the Earth Europe Biotechnology Programme has a new Web page. Besides introducing its programme and goals, it includes the last five issues of their very useful *FoEE Biotech Mailout*. FoEE has for some years closely monitored the political chess game involving GMOs in Europe. This new method of outreach, in addition to its paper and e-mail versions of its Biotech Mailout, is most welcome.
http://www.foeeurope.org/programmes/biotechnology/about.htm

More and more companies are selling information on biotechnology on the Internet. As bait, many of them offer bites of information that can be regularly chased by information hunters. If you are one of those, here are some of these commercial information sources:

- *Genetic Engineering News* mainly traces the pharma-biotech sectors, although it also covers agbiotech. The

abstracts of the current issue's articles and a list of former ones are also on the Web page.
http://www.genengnews.com

- Newspage is a company that provides personalized news-sheets through which one can receive information on selected issues or companies, such as Agricultural Biotechnology, Biotechnology Business News, Shaman Pharmaceuticals or Monsanto. Some of the information is only "pay per view", yet there is still a lot which can be accessed for free or at low cost. Really useful.
http://www.newspage.com/NEWSPAGE

(Source: *Seedling*, March 1998)

If you are interested in finding out the opinion of those affected by the main illnesses biotech promoters hold promise to cure, it will be worth a visit to the Euthanasia and biotechnology home page. Beware of the link to the *Future Generations* Web page, which is between pirate skulls: the contents of the Web page calling for eugenics are extremely distasteful.
http://www.thalidomide.ca/gwolbring/

The Council for Responsible Genetics, which publishes the newsletter *GeneWATCH*, a US NGO that deals with biotechnology, has a strong campaign against life patenting and genetic discrimination. Its Web page contains position statements on genetic discrimination, cloning, patenting of life forms and genetically engineered foods, and very useful briefing documents that analyse the main issues around life patents, the genetic determinism underpinning genetic research, and genetic privacy. It is a pity that the site does not offer *GeneWATCH* on line, even for back issues, and that it was last updated in August 1997. Otherwise, it is worth a visit.
http://www.essential.org/crg/

In its issue of 9 April, *Nature* published a special report on bioprospecting. Although the report did not question the benefits of life patenting, it at least made an effort to include the so-called "alternative" approach. Both the report articles and a selection of Web sites dealing with bioprospecting (not particularly comprehensive) can be found at *Nature*'s Web page, in the "Web Specials" section.
http://www.nature.com/

The International Rice Research Institute (IRRI) has set up a useful service on rice: the *Riceweb*. This provides extensive data on things like the main rice production systems, the most important producers and consumers and the newest research an development. Of course, there is not much information on genetic erosion or criticism of modern breeding techniques, but it is useful all the same.
http://www.cgiar.org/irri/riceweb
(Source: *Seedling*, June 1998)

BIO-IPR: A new GRAIN listserver!

GRAIN has just launched an electronic listserver: BIO-IPR. Its purpose is to circulate information about recent developments in the field of intellectual property rights related to biodiversity and associated knowledge. To get on the mailing list, send the word "subscribe" (no quotes) as the subject of an e-mail message to bio-ipr-request@ cuenet.com. To get off the list, send the word "unsubscribe" instead. To submit material to the list, address your message to bio-ipr@cuenet.com. A note with further details about BIO-IPR is sent to you as soon as you subscribe. You can also subscribe via our homepage: http://www.grain.org, where you will also find lots of other interesting material.

GRAIN: Girona 25, pral, E-08010, Barcelona, Spain. Tel.: (34-93) 301 13 81; fax: (34-93) 301 16 27; e-mail: grain@bcn.servicom.es Web: http://www.grain.org (Source: *Seedling*, September 1998)

Genetech on net

Austria's consumer protection minister, Barbara Prammer, unveiled a new genetech listing as required under a new Austrian law on the Internet. The law requires that all the genetically-altered products available on the market are listed clearly.

The address is http://www.gentechnik.gv.at. The page lists the name of the product and says when the product was first registered, what kind of genetic manipulation was made, and the exact labelling of the product.

The producer, importer and how to use it, including what it is good for, are also listed. General legal implications surrounding the product are also listed. Austria has the strictest controls on the use of genetically-altered foodstuffs and products in the EU, and as a result, just 13 items are listed on the Internet page. Experts say that eventually Austria will be forced to liberalize its market in line with other EU countries, and by then the consumer protection department believes that the list will be far more useful to people concerned about avoiding genetically-altered products. (Source: *Austria Today*, 9 July 1998)

Food safety explained on the AAS Internet

To help the public learn more about food safety, the Australian Academy of Science has added a new topic to its Nova:Science in the news Internet site (http://www.science.org.au/nova). Called "When bugs have you on the run", the topic is supported by the Cooperative Research Centre for International Food Manufacture and Packaging Science. (Source: *Australasian Biotechnology*, vol. 8, No. 4, August 1998)

Royal Society of Chemistry databases launched on ChemWeb

ChemWeb, In. (http://ChemWeb.com) and the Royal Society of Chemistry (http://www.rsc.org) are pleased to announce the launch of four more RSC databases on ChemWeb. ChemWeb members can choose between pay-per-view or subscription payment methods.

Chemical Business NewsBase is a vital source of information for all those in the chemical industry, providing highly current facts and figures on company activities and results, new products, markets, legislation, environmental aspects, and new technologies and trends. This major business information database is updated twice weekly. More than 1,300 new items are added each week.

Chemical Engineering and Biotechnology Abstracts is a collaboration of The Royal Society of Chemistry, the DECHEMA, FIZ CHEMIE and IchemE, formed to produce the foremost database in chemical engineering. CEABA provides worldwide information in the fields of chemical and process engineering, safety engineering, environmental protection, corrosion, materials science and biotechnology.

Chemical Safety NewsBase provides information on the health and safety issues affecting the chemical and allied industries, the laboratory and the office environment. Subject

coverage includes: chemical reactions; biological hazards; safety precautions; legislation; fires and explosions; waste management and storage practices; emergency planning and office hazards.

Mass Spectrometry Bulletin is the most comprehensive current awareness publication in mass spectrometry. It contains titles, bibliographic details and keywords for recently published documents dealing with all areas of mass spectrometry and related ion processes. These are selected from over 800 primary journals, conference proceedings, new books, patents and reports.

Analytical Abstracts, added recently to ChemWeb, now has the complete backfile available. Data back to 1970 can now be searched on ChemWeb. (Source: *Australasian Biotechnology*, vol. 8, No. 4, August 1998)

Annotated List of US Web Sites

Information Systems for Biotechnology

ISB provides information on agricultural and environmental biotechnology research, product development, regulatory issues and biosafety. This service is supported by a grant from USDA's Cooperative State Research, Education and Extension Service to Virginia Tech.
http://www.nbiap.vt.edu

National Agricultural Library

NAL provides access to a variety of information services and publications covering many aspects of agricultural biotechnology. Specific topics include theory and techniques of genetic engineering, plant and animal genetics, monoclonal antibodies, single cell proteins, food processing, biomass applications and risk assessment and bioethics.
http://www.nal.usda.gov/bic

USDA/APHIS Biotechnology Permits

A division of USDA's Animal and Plant Health Inspection Service, Biotechnology and Scientific Services regulates and issues permits for the importation, interstate movements and environmental release of certain genetically engineered plants and micro-organisms under the Code of Federal Regulations, vol. 7, part 340.
http://www.aphis.usda.gov/bbep/bp

National Institutes of Health Office of Recombinant DNA Activities

This office is responsible for the "NIH Guidelines for Research Involving Recombinant DNA Molecules".
http://www.nih.gov/od/orda

USDA/APHIS Transgenic Arthropod Team

This was formed to deal with questions and policy related to transgenic arthropods and other invertebrates.
http://www.aphis.usda.gov/bbep/bp/arthropod

U.S. EPA Toxic Substances Control Act, Office of Pollution Prevention and Toxics

This site was created to allow more efficient public, governmental and educational access to the TSCA Biotechnology Program. Here you will find the regulation under which the Programe functions, supplementary documents created to support this regulation, as well as status reports on the submissions, reviews and agreements undertaken by the Program. http://www.epa.gov/opptintr/biotech

U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition

Proposed, pending and final rules and policies regarding food biotechnology.
http://vm.cfsan.fda.gov/~lrd/biotechm.html

USDA Biotechnology Risk Assessment Research Grants

Abstracts for the 1996 Grant Awards are available, as well as Program contact information. Future Solicitations for Proposals will also be posted here.
http://www.reeusda.gov/crgam/biotechrisk/biotech.htm

Institute for Biotechnology Information

IBI has provided comprehensive information on commercial biotechnology to hundreds of organizations worldwide—corporations, universities and government agencies. IBI makes this information available in a variety of formats, including databases, a unique array of publications and special studies.
http://www.biotechinfo.com

National Biotechnology Information Facility

The National Biotechnology Information Facility (NBIF) will provide a single-point access to a vast store of widely distributed biotechnology data. NBIF will encourage information-sharing between researchers, promote biotechnology in Historically Black Colleges and Universities/ Minority Institutions and provide training in biotechnology. NBIF will also be active in developing new sources and types of biotechnology databases.
http://www.nbif.org

National Agricultural Biotechnology Council

Providing people with differing viewpoints a neutral forum where they can come together and freely exchange ideas on the critical issues facing agricultural biotechnology, the National Agricultural Biotechnology Council (NABC), founded in 1988, counts among its membership the leading agricultural research and educational institutions from throughout the US and Canada.
http://www.cals.cornell.edu/extension/nabc

Union of Concerned Scientists

The common threads of global sustainability and global security weave the Union of Concerned Scientists' work on agriculture, arms control, energy, global resources and transportation into a unified vision: achieving a secure and sustainable world today without sacrificing the environment of tomorrow. http://www.ucsusa.org

Institute of Food Technologists

Founded in 1939, the Institute of Food Technologists is a non-profit scientific society with 28,000 members working in food science, food technology, and related professions in industry, academia and government. http://www.ift.org

Biotechnology Industry Organization

The Biotechnology Industry Organization (BIO) is the largest trade organization to serve and represent the emerging biotechnology industry in the US and around the globe. The organization's leadership and service-oriented guidance have helped advance the industry and bring the benefits of biotechnology to the people of the world.
http://www.bio.org

Bioportfolio

Dedicated to increasing awareness and communication between biotechnology investors, researchers and industry, Bioportfolio specializes in applied commercial developments utilizing modern biotechnological techniques impacting on the life sciences and associated industries.
http://www.bioportfolio.com

The Food Biotechnology Center

A clearinghouse for information, with industry and the University pointing to produce outstanding research and practical knowledge. Their mission is to develop competitive, highly integrated food production systems that provide safe, economical food products through the utilization of molecular tools and methods. http://fabctr.umn.edu

Progressive Farmer Online—Biotechnology

The future of farming is here—seeds genetically designed to fight pests and resist herbicides. Progressive Farmer Online takes a look at what this new technology means and what is on the biotechnology horizon.
http://www.pathfinder.com/@@XteY9gQA7hO6mHbX/PF/features/biomenu.html

Public Perception Issues in Biotechnology

The goal of this Web site is to provide information on communication of biotechnology issues and the impact of biotechnology on society. The material currently displayed covers public perceptions of the scientific, regulatory, educational, and commercial issues involving the many different aspects of biotechnology, with emphasis on the environment and agriculture.
http://fbox.vt.edu:10021/cals/cses/chagedor/index.html

National Genetic Resources Program

The Germplasm Resources Information Network (GRIN) Web server provides germplasm information about plants, animals, microbes and invertebrates within the National Genetic Resources Program of the US Department of Agriculture's (USDA) Agricultural Research Service (ARS).
http://www.ars-grin.gov

Agricultural Genome Information System

AGIS provides genome information for agriculturally important organisms. At present, this encompasses mostly crop and livestock animal species. Also included are a number of databases that have related information, e.g. germplasm and plant gene nomenclature data. AGIS is a cooperative effort between the Department of Plant Biology, University of Maryland, and USDA's National Agricultural Library's Genome Informatics Group.
http://probe.nalusda.gov:8000/

American Genetic Resources Alliance

A diverse group which is interested in the conservation of plant genetic resources. It evolved from a focus group concerned with the work of the USDA/ARS National Plant Germplasm System, and is working to generate public awareness of and support for the National Plant Germplasm System's genetic resource conservation work.
http://www.amgra.org/index.htm (Source: *Australasian Biotechnology*, vol. 8, No. 4, August 1998)

BioGroup

The Bioremediation Discussion Group (BioGroup) home page (http://biogroup.gzea.com) consists of a moderated Internet mailing list serving approximately 1,700 members worldwide. The BioGroup was established to provide a global forum for the environmental science/engineering communities to discuss intrinsic/enhanced bioremediation topics. The home page contains information about bioremediation, the BioGroup mailing list, a searchable archive of technical papers that may be downloaded at no cost, and links to more than >900 related Web resources. (Source: *Australasian Biotechnology*, vol. 8, No. 3, June 1998)

Molecular Modeling Electronic Conference

The Molecular Modeling Electronic Conference (TMMeC) announces that the papers on display for its current number are now open for discussion. TMMeC is accessible at: http://bilbo.edu.uy/tmmec/

TMMeC is a non-profit, educational activity sponsored by the Universidad de la República, Montevideo, Uruguay. TMMeC is a freely accessible, index (ISSN 0797-9274), peer-reviewed multimedia publication established with the aim of providing a setup for fast and continuous display of current work in molecular modelling and computational chemistry. (Source: *Australasian Biotechnology*, vol. 8, No. 3, June 1998)

Biotechnology Industrial Platforms

The Biotechnology Industrial Platforms (IPs) have set up a combined Web page site. It provides details on their aims and members as well as contacts. These platforms are a feature of the EU's Biotechnology Programme and are industrial groupings established around specific research topics with a primary objective of exploiting EU-funded biotechnology R&D. Since the first platforms were established in 1990, the number has now grown to 11, with the latest platform member being the Healthy Ageing Europe 2000 (HAE 2000). The Web site address is: http://europa.eu.int/comm/dg12/biotech/ip.html (Source: *Australasian Biotechnology*, vol. 8, No. 3, June 1998)

Australian Academy of Technological Sciences & Engineering

The site may be accessed at http://www.atse.org.au. It is being developed as an additional means for communicating with the diverse spectrum of audiences important to the Academy. (Source: *Australasian Biotechnology*, vol. 8, No. 3, June 1998)

Current Contents Connect

Take a free trial or subscribe to Current Contents Connect: http://connect.isihost.com. There are seven multi-disciplinary editions: Life sciences; Clinical medicine; Engineering, Computing and Technology; Agriculture, Biology and Environmental Sciences; Physical, Chemical & Earth Sciences; Social & Behavioral Science; Arts & Humanities. (Source: *Australasian Biotechnology*, vol. 8, No. 3, June 1998)

Organisation for Economic Cooperation and Development Biotrack Online

BioTrack Online is part of OECD's Programme on the Harmonization of Regulatory Oversight in Biotechnology. The main aim of this Programme is to produce documents for use by national authorities for the regulatory assessment of genetically modified or release to the environment. The focus is on genetically modified plants and micro-organisms. Home

of BIOTRACK, a database of environmental releases from OECD member countries.
http://www.oecd.org/ehs/projects.htm

Biosafety Information Network and Advisory Service

BINAS is a service of the United Nations Industrial Development Organization (UNIDO). BINAS monitors global developments in regulatory issues in biotechnology. It tracks regulatory developments from many countries, including the full text of regulations and guidelines.
http://binas.unido.or.at/binas/binas.html

OECD's Biotrack and BINAS

A joint link-up between OECD's Web site and UNIDO's BINAS Web site, both listed here. This is an alternative method for connecting to either Web site.
http://www.olis.oecd.org/biotrack.nsf

United Nations Environment Programme International Register on Biosafety

This Web site offers information from many sources on biosafety. It focuses on information useful in establishing a regulatory framework for the safe development, transfer and application of biotechnology. It also provides links to other Web sites concerning biosafety, biotechnology and biodiversity. http://irptc.unep.ch/biodiv

Biosafety Research and Assessment of Technology Impacts of the Swiss Priority Programme

The BATS agency was founded in 1993 as a core project of the Priority Programme Biotechnology of the Swiss National Science Foundation. The BATS agency is active in the acquisition, processing and communication of application-oriented information and know-how in biotechnology. http://www.eurospider.ch/BATS/index.html

Advisory Committee on Releases to the Environment/ Advisory Committee on Genetic Modification (UK)

ACRE and ACGM give advice to the Secretary of State on human and environmental safety concerning the releases of genetically modified and non-native organisms into the environment. http://www.shef.ac.uk/~doe

International Centre for Genetic Engineering & Biotechnology (ICGEB) (Italy)

ICGEB is an international organization established to promote the safe use of biotechnology worldwide, with special regard to the needs of the developing world. ICGEB coordinates a network of national laboratories in member countries. ICGEB's main activities are research, training (fellowships, courses) and scientific services. The research activities are carried out by the laboratories of ICGEB, and also at the affiliated centres, through a system of collaborative research grants. http://base.icgeb.trieste.it

Biotechnology Strategies and Coordination Office (Canada)

BSCO was formally established in 1993 (although it has been in operation since 1988) to provide a one-window approach for information on biotechnology in Agriculture and Agri-Food Canada.
http://www.cfia-acia.ca/english/food/biotech/bsco.html

Belgian Biosafety Server

This covers Biosafety in Belgium, Biosafety in Europe, Biosafety in the World, General Biosafety, Recommendations, The Service of Biosafety and Biotechnology.
http://biosafety.ihe.be

Agricultural Biotechnology for Sustainable Productivity

This is funded by the US Agency for International Development to enhance institutional capacity for the use and management of agbiotech research. Its objectives are to address insect and pathogen constraints to food crop production in developing countries, to develop bioreactor micropropagation technology for cloning high-value plantation crops, and to foster policy implementation which ensures an environmentally and socially responsible transfer of the technologies and their products.
http://www.css.msu.edu/users/sa/absp.htm

Convention on Biological Diversity—Working Group on Biosafety

The Open-ended Ad Hoc Working Group on Biosafety (BSWG) held its first meeting in Aarhus, Denmark, from 22 to 26 July 1996 to begin the elaboration of a global protocol on safety in biotechnology. More than 90 delegations, including scientific and technical experts, representing both Parties and non-Parties to the Convention on Biological Diversity (CBD) attended the meeting, as did observers representing intergovernmental organizations, NGOs and industry. http://www.iisd.ca/linkages/biodiv/bios

Innovative Technology of the Ministry of Agriculture, Forestry and Fisheries (Japan)

This site provides guidelines, current status field tests, releases and commercialization of transgenic plants.
http://ss.affrc.go.jp/docs/sentan

The European Federation of Biotechnology Task Group on Public Perceptions of Biotechnology

Established in 1991, some 50 members have been brought together from a wide range of groups with interests in biotechnology from across Europe. Its primary aim is to foster greater public awareness and understanding of biotechnology and to encourage public debate.
http://www.kluyver.stm.tudelft.nl/efb/tgppb/home.htm

International Service for the Acquisition of Agri-biotech Applications

ISAAA, the International Service for the Acquisition of Agri-biotech Applications, is a non-profit international organization co-sponsored by public and private sector institutions with the aim of facilitating the acquisition and transfer of agricultural biotechnology applications from the industrial countries, particularly proprietary technology from the private sector, to developing countries for their benefit.
http://www.isaaa. cornell.edu

Cassava Biotechnology Network

The Network's small-grants program and regional representatives encourage international and interdisciplinary cooperation. A newsletter, directory and international meetings keep cassava biotechnology specialists in touch.
http://www.ciat.cgiar.org/cassava/cbn/cbn.html

International Rice Research Institute

IRRI is a non-profit agricultural research and training centre established to improve the well-being of present and future generations of rice farmers and consumers, particularly those with low incomes. It is dedicated to helping farmers in developing countries produce more food on limited land using less water, less labour and less chemical inputs, and without harming the environment.
http://www.cgiar.org:80/irri

The Virtual Center of Biotechnology for the Americas

This site provides fast and convenient means for receiving and exchanging biotechnology-related information with particular emphasis on issues affecting Latin America.
http://www.ibt.unam.mx/virtual.cgi

IRRO Databases on Environmental Releases

Information Resource for the Release of Organisms to the Environment (IRRO) is an information network run on a non-profit basis, which aims to provide access to all types of information relevant to the release of animals, plants and micro-organisms into the environment.
http://www.bdt.org.br/bdt/irro

Cooperative Research Centre for Plant Science (Australia)

The Centre exploits plant biotechnology to develop and apply plant molecular, cellular and physiological research and training to the agri-food and fibre industries and public sector.
http://biology.anu.edu.au/CRCPlantScience.html

International Service for National Agricultural Research

ISNAR assists developing countries in bringing about lasting improvements in the performance of their national agricultural research systems and organizations. It does this by promoting appropriate agricultural research policies, sustainable research institutions and improved research management. ISNAR's services to national research are ultimately intended to benefit producers and consumers in developing countries and to safeguard the natural environment for future generations. http://www.cgiar.org/isnar

Agricultural Biotechnology Centre (Hungary)

The Centre deals with biotechnology research and development for an environmentally friendly Hungarian agriculture. Its main tasks are research by means of genetic engineering and cellular techniques, development of the technological and technical level of agriculture with environmentally adequate methods, and the assurance of conditions for the transfer of new technologies. http://www.abc.hu

CGIAR Research Centers

This site provides links to the 16 International Agriculture Research Centers of CGIAR, the Consultative Group on International Agricultural Research.
http://www.cgiar.org:80/centers.htm

Base de Dados Tropical

The Base de Dados Tropical (Tropical Database) is a department within the Fundação Tropica de Pesquisas e Tecnologia "Andre Tosello", a Brazilian non-profit, private foundation. This bioinformatics facility provides access to databases as well as to many other Internet resources.
http://www.bdt.org.br/bdt

BIOSIS

This is an electronic public debate about biotechnology. Of particular interest is the Commercialization of Plant Biotechnology Products. http://www.scicomm.org.uk/biosis

Bioline's Online Journal Biosafety

Titles and abstracts of journals are available for browsing or searching free of charge. Full text and associated graphics of material of interest may be requested online, following registration.
http://www.bdt.org.br/bioline/by

DNA Bank at MAFF (Japan)

DNA Bank at the Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan is responsible for the preservation of DNA and molecular information on agricultural organisms. This bank acts as a research support service.
http://bank.dna.affrc.go.jp

Fralin Biotechnology Center at Virginia Tech

The Fralin Center serves as a focal point for the development and dissemination of information of the techniques and disciplines that make up this quickly evolving field. The Center serves to foster and coordinate interdisciplinary research efforts in order to learn, to teach, and to reach out to our community, to Virginia, and to the world.
http://www.biotech.vt.edu

Center for Plant Biotechnology Research—Tuskegee University

The primary mission of this Center is to employ innovative molecular and cellular genetic tools in the improvement of select crops such as sweet potato, peanut, cowpea and muskmelon, and to provide training in plant biotechnology to ethnically under-represented minority students and scientists from developing countries.
http://agriculture.tusk.edu/AgHe%20Website/Biotech%20Website/biotech.html

University of Wisconsin—Biotechnology Center

The mission of the University of Wisconsin Biotechnology Center is to maximize the benefits of biotechnology to the University of Wisconsin-Madison, University of Wisconsin System, state and nation by being an excellent quality, comprehensive, multidisciplinary biotechnology centre that supports, coordinates, disseminates and advances biotechnology. http://calvin.biotech.wisc.edu

University of Idaho and IMAGE

The Institute for Molecular and Agricultural Genetic Engineering (IMAGE) was established at the University of Idaho in 1984 to promote and coordinate biotechnology research. Projects of interest to IMAGE have been in the areas of hazardous waste bioremediation, fermentation technology, microbial ecology and animal biotechnology. The Plant Biotechnology Group was recently established under the sponsorship of IMAGE, to increase the University's effort in this important area. http://image.fs.uidaho.edu (Reproduced from *ISB News Report—January 1998*) (Source: *Australasian Biotechnology*, vol. 8, No. 5, October 1998)