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A. SPECIAL ARTICLE

OVERVIEW OF PESTICIDES AND PCBS REMEDIATION TECHNOLOGIES*

By

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Abstract

A review of established and emerging technologies for treatment of pesticides and polychlorinated biphenyls (PCBs) is presented. The technologies are classified in biological, physico-chemical and thermal treatments describing main unit operations and comparing technical, social and environmental limitations, including some potential risks and environmental impacts. Estimated overall costs, clean-up times, reliability and maintenance levels are also presented in order to assess advantages and limitations of each technology.

Keywords: Pesticides, PCBs, remediation, waste, soil, treatment.

1. Introduction

The purpose of this paper is to review the existing technologies to treat pesticides and polychlorinated biphenyls (PCBs), presenting their limitations and some technical, environmental, social and economic criteria to choose the most proper technique. The first part describes general properties of pesticides and PCBs that have been classified among POPs (Persistent Organic Pollutants), an increasing environmental problem that has enhanced the research and development of different technologies to control and prevent the impact generated by these compounds. This paper is mainly focused on remediation technologies developed to address the problems generated by pesticides and PCBs already produced; technologies to prevent pesticides and PCBs effects are not included on this review.

Some criteria are presented on Remediation Technologies (section 3), including overall costs, clean-up time, reliability and maintenance. The technologies have been classified as "established" and "emerging/innovative technologies", in order to differentiate between those technologies having demonstrated full-scale applications and those having few full-scale applications but proven efficiencies in pilot and laboratory scales.

Every section is divided on physico-chemical, thermal and biological techniques, although some technologies are combinations of thermal-chemical or thermal-biological methods. Each technology is described based on operational conditions and unit operations; the main limitations of each technology are also presented and classified as technical/economical, social

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and environmental (including environmental impacts and safety risks). The list of technologies discussed is not exhaustive since many technologies are currently being developed and others are variations of existing ones.

Finally some recommendations and conclusions are presented to stress the importance of considering both ratable and non-ratable criteria during the selection of technologies, as well as social, technical, environmental and economical parameters, which must be considered while assessing and performing pesticides and PCBs remediation projects.

2. Generalities

POPs (Persistent Organic Pollutants) are highly stable organic compounds used as pesticides, herbicides, fungicides, or in chemical industry. They are also generated as the byproduct of combustion and industrial processes. They persist in the environment, accumulate in the fatty tissues of living organisms and are toxic to humans and wildlife. POPs are typically semi-volatile, enabling them to move long distances and condense over colder regions of the planet. They are classified according to lipophilicity, persistence (resistance to photolytic, chemical and biological degradation) and toxicity.

The Convention on Long-Range Transboundary Air Pollution (LRTAP) has defined criteria and procedures for adding substances to the Protocol on Persistent Organic Pollutants. A party proposing to add a substance must provide the LRTAP Executive Body with a risk profile on that substance and information related to these four characteristics (USEPA, 1998):

- The potential for long-range transboundary atmospheric transport: vapour pressure below 1,000 Pa and an atmospheric half-life greater than two days or monitoring data that evidence the substance is found in remote regions.
- Persistence: a half-life in water greater than two months, soil and sediment half-lives greater than six months or, alternatively, evidence that the substance is otherwise sufficiently persistent to be of concern.
- Bioaccumulation: evidence that the fish bioaccumulation factor is greater than 5,000 or the log Kow is greater than 5 or, if those values are not achieved, other factors that could make the substance of concern.
- Toxicity: potential to affect human health and/or the environment.

After a technical review, the parties to the Protocol meeting within the LRTAP Executive Body decided by consensus whether a substance is within the scope of the Protocol and whether to adopt the proposal to change the Protocol to add that substance. The substances scheduled for elimination according to the Protocol to the Convention of Long-Range Transboundary Air Pollution on POPs are listed at first in Table 1, as well as other pollutants considered as POPs; note that 10 of the 12 main pollutants are pesticides.

3. Remediation technologies

Waste and soil remediation technologies can be classified according to their development status. "Established technologies" are those having demonstrated full-scale applications and removal efficiencies; "emerging technologies" refer to methods having few full-scale applications but proven pilot- and laboratory-scale removal efficiencies; finally, "innovative technologies" are those with no reported full-scale applications, but with removal efficiency proven at laboratory and pilot scales.

Tables 2 and 3 present some criteria that can be considered to select a remediation technology; the overall cost includes design, construction, operation and maintenance cost of the technology; it does not include previous assessments or post treatment costs, nor manpower. The reliability and maintenance criteria refer to the level of process complexity and the ease to maintain.

Although other criteria should be considered when selecting a remediation technology, such as the technique's ability to clean to an acceptable level (minimum pollutant concentration achievable by the technology), community acceptability, applicability, post treatment costs, agricultural soil use after treatment, environmental impacts and risks of remediation activities/processes.

3.1 Established technologies

3.1.1 Physico-chemical technologies

Landfill cap system

Landfill capping is one of the most common forms of remediation technologies. It is used to cover buried waste materials to prevent contact with the environment and to effectively manage the human and ecological risks associated with a remediation site. The design of landfill caps is specific and depends on the intended functions of the system. The most critical components of a landfill cap are the barrier and the drainage layers. 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 needed in a wet climate; the system complexity also depends on the type of waste (phase, hazardous or solid waste). The materials used in the construction of landfill caps include low- and high-permeability soils and low-permeability geosynthetic products: The low-permeability material drains water and prevents its passage into the waste. The high permeability materials collect the water that percolates into the cap.

Landfill caps may be temporary or permanent. Temporary caps can be installed before permanent closure to minimize generation of leachate until a better remedy is selected. These caps are usually used to minimize infiltration when the underlying waste mass is undergoing

Table 1. List of POPs

Aldrin (pesticide)	Dieldrin (pesticide)	Hexachlorobenzene, HCB (pesticide)
Chlordane (pesticide)	Endrin (pesticide)	Mirex (pesticide)
Chlordecone (pesticide)	Heptachlor (pesticide)	Polychlorinated biphenyls (PCBs)
DDT (pesticide)	Hexabromobiphenyl	Toxaphene (pesticide)
Other pollutants		
Dioxins and furans	Polychlorinated naphtalenes	Polycyclic aromatic hydrocarbons
Polychlorinated benzenes	Polychlorinated paraffins	Kepone (chlordecone)
Polychlorinated phenols	Polybrominated compounds	Lindane
Isodrin	Parathion	Malathion

Table 2. Some criteria to assess established remediation technologies

Remediation technology	Overall cost* (USD/ton)	Clean-up time	Reliability and maintenance (level)
Physico-chemical			
Landfill cap system (in or ex)	N.A.	-	Varies
Vapour extraction (in)	S	M to L	Average
Vapour extraction (ex)	S	M to L	High r. and Low m.
Thermal technologies			
Combustion systems (ex)	M to L	S to M	Average
Thermal desorption (in or ex)	S to M	S to M	Average
Pyrolysis (ex)	M to L	S	Average
Biological technologies			
Bioventing (in)	***M***	M to L	Low r. and High m.
Composting (ex)	M	M to L	Average
Biopiles (ex)	S	S	Average
Land farming (ex)	S	M to L	High r. and Low m.

Table 3. Some criteria to assess innovative and emerging remediation technologies

Remediation technology	Overall cost * (USD / ton)	Clean-up time	Reliability and maintenance (level)
Physico-chemical			
Base catalyzed dechlorination (ex)	M to L	S	Average
Electrochemical oxidation (in)	M to L	S to M	Low r. and high m.
Solvent extraction (ex)	M to L	S to M	Average
Solvated electron (ex)	L	S	Average
Supercritical water oxidation (ex)	S to M	S	Average
Solar detoxification (ex)	N.A.	S to M	N.A.
Gas phase chemical reduction (ex)	L	S to M	High m.
Catalytic hydrogenation (ex)	N.A.	S	High r. and low m.
Thermal technologies			
T. D. – Catalyzed dehalog. (ex)	M to L	S	Low r. and high m.
T.D. – Pyrolysis (ex)	L	S	Average
T.D. – Retort system (ex)	M to L	S to M	Average
Plasma ARC Systems (ex)	L	S	Average
Vitrification (in or ex)	M to L	S	High r. and low m.
Biological technologies			
Bioslurry (ex)	M	S to M	Average
Enhanced bioremediation (in)	S	L	Low r. and high m.

(in) = in situ (ex) = ex situ; S = short term < 6 months, M = medium, 6 to 12 months, L = long, > 12 months.
*Cost (USD): S = less than $150, M = $150 to $300, L = more than $300;
r. = reliability; m. = maintenance. T.D. = thermal desorption. N.A. = not available.

Table 4. Main limitations of established physico-chemical technologies

	Landfill cap systems	Vapour extraction (SVE)
Technical/economical	Toxicity is not reduced and pollutants are not destroyed by these methods.	Low permeabilities, high humidity content and soil heterogeneity limit performance. The method is only suitable for medium to high volatile compounds.
Social	In some cases this method may attract public opposition.	Usually does not attract public opposition.
Environmental	Precautions must be taken to ensure the cap is not damaged by land use activities. PCBs and other semivolatile pollutants may evaporate more rapidly with increased moisture in soils and sediments (Chiarenzelli, 1998). Potential leaking of hazardous compounds.	Potential releases of hazardous compounds during excavation and materials handling. Exhaust air from SVE requires secondary treatment.

settling. A more stable base will thus be provided for the final cover, reducing the cost of the post-closure maintenance. Landfill caps can also be applied to waste masses too large for other treatments. Disposal in a landfill is not a proper method for liquid pesticides or highly mobile waste. Inorganic pesticides or liquid pesticide waste containing about five per cent organic material can be solidified or stabilized prior to disposal in a landfill (USEPA, 1999).

Vapour extraction

Soil vapour extraction (SVE) is a well-established, economic and efficient technique for the removal of volatile organic compounds (VOCs) and halogenated organics (including pesticides and PCBs). The technology can be used for treating contaminants *in-situ* or *ex-situ*. *In-situ* SVE uses a vacuum system that uses extraction wells to create a concentration gradient that enhances gas phase volatiles removal from soil through the extraction wells. During *ex-situ* SVE remediation the excavated soil is placed over a network of aboveground piping where vacuum is applied to encourage volatilization of organics. The soil piles may be sealed with geomembranes to avoid volatile emissions and soil saturation due to lixiviation. This technique has an advantage over *in-situ* methods as a result of increased passageways, being able to collect leachate, and making possible a more uniform treatment (UNECE, 1997).

3.1.2 Thermal technologies

Combustion systems

High temperature incineration

This has been one of the most applied remediation technologies for the treatment of a variety of contaminant sources including pesticides, PCBs and explosives. It is a high temperature (870° C to 1,200° C) destructive *ex-situ* treatment of polluted soil; the waste and/or contaminated soil are fed into the incinerator under controlled conditions, the high temperatures in the presence of oxygen volatilize and combust the contaminants into innocuous substances. Although a variety of designs are available, most incinerator designs are fitted with rotary kilns, combustion chambers equipped with an afterburner, a quench tower and an air pollution control system. Removal efficiencies of more than 99.99 per cent are feasible. For PCBs and dioxins, the high temperature incinerators can achieve destruction and removal efficiencies of up to 99.9999 per cent (OHMRS, 1995).

Modern incinerators are commonly described as destroying pesticides, PCBs and similar chemicals very efficiently. However, recent tests suggest that incinerators achieve destruction efficiencies that are lower than those achieved by certain non-combustion technologies. In addition, some incinerators burning POPs (pesticides and PCBs) and other waste are associated with the spread of undestroyed and newly formed POPs (dioxins and furans) into the surrounding environment, contaminating the air, soil, vegetation, wildlife and human populations (Costner, 1998).

The USEPA has approved high efficiency incinerators to destroy PCBs with concentrations above 50 ppm. Incinerators destroying PCB liquids must meet technical requirements like 2-sec residence time at 1,200° C and 3 per cent of excess oxygen, alternatively, 1.5-sec residence time at 1,600° C and 2 per cent of excess oxygen in the stack gases. The destruction and removal efficiency (DRE) for non-liquid PCBs must be equivalent to 99.9999 per cent (less than 1 ppm).

Cement kilns

The main processes employed in making cement clinker can be classified as either "wet" or "dry", depending on the method used to prepare the kiln feed. In the wet process the feed material is slurried and fed directly into the kiln. In the dry process the kiln exhaust gases are used to dry raw material while it is being milled.

At the very high temperature of the cement kiln, and with the long residence times available, very high destruction efficiency is possible for hazardous waste. The highly alkaline conditions in a cement kiln are ideal for decomposing chlorinated organic waste. Chlorinated liquids, chlorine and sulphur are neutralized in the form of chlorides and sulphates. The quantities of inorganic and mineral elements added in treating chlorinated waste are limited (usually a small fraction of the large feed requirements of a commercial kiln). No liquid or solid residues requiring disposal are generated since all residues are bound within the product.

The most appropriate waste for disposal in cement kilns are those which provide additional energy value as a substitute fuel, or material value as a substitute for portions of the raw material feed (e.g. calcium, silica, sulphur, alumina or iron). Liquid waste or low ash waste can be relatively easy to burn in cement kilns. The material is fed in dry or in slurry form (especially for the "wet" process), or as a fuel supplement into the burning zone of the kiln. In this zone, the temperature of 1,450° C is able to perform high destruction efficiency as the gas passes though the kiln.

For the typical counter current process configuration, polluted soils and solid waste cannot be fed into the firing end of the kiln, since they would discharge in the clinker without adequate treatment; besides, they cannot be fed into the cool end of the kiln, as the waste would volatilize and would not be adequately destroyed. There are two suitable options for feeding the waste. The first one consists of feeding solid material at the middle of the kiln through a specially designed hopper; the kiln temperature at feeding point is approximately 1,100° C and increases as the materials pass further down the kiln This involves a major modification to the rotary kiln Monitoring and verification is required that a complete destruction of stable chlorinated compounds such as PCBs occurs with the desired efficiency (Hansen, 1992).

Table 5. Main limitations of established thermal technologies

	Combustion systems	Thermal desorption	Pyrolysis
Technical/economical	Require cleaning systems for heavy metals. Need strict control to prevent dioxins formation. Older types of cement kilns are not suitable.	Require dewatering to achieve proper soil moisture levels. Must be linked to a post treatment.	Does not attack inorganic compounds. Performance depends on the soil moisture content, which has correlation with overall cost.
Social	In many cases may attract public opposition.	May present public opposition if linked to combustion systems.	Usually does not attract public opposition.
Environ-mental	Emission of combustion products. Potential release of toxic compounds (dioxins, furans, chlorinated compounds).	Potential of fugitive emissions. Emission of combustion gases and potential formation of dioxins (when linked to combustion systems).	Require controls and systems to prevent dioxins formation. Needs control of combustion gases.

The second option includes a pretreatment of the solid waste (e.g. thermal desorption, such as the approach taken with catalyzed dehalogenation systems). After such treatment the material can be utilized as a raw material substitute, and the condensate can be incorporated in the liquid feed stream.

When operated properly, destruction of chlorinated compounds in cement kilns can be >99.0000 per cent complete with no adverse effect on the quality of the exhaust gas (Benestad, 1989). The contribution of waste materials to the exhaust gases is relatively minor, given that the wastes are only used as a minor supplement to the main energy or raw material stream.

Thermal desorption

Thermal desorption is an *ex-situ* process to remove volatile and semi-volatile contaminants that are sorbed on the waste, by heating to temperatures (between 170 to 550° C) high enough to volatilize the organic contaminants. Thermal desorption is not a stand-alone technology and must be followed by a subsequent method to treat the off-gas (which is normally captured by a carrier gas or vacuum system) in order to remove particulates and contaminants. Wet scrubbers or fabric filters are one of the best units to remove particulates while contaminants are removed through condensation followed by carbon adsorption, a secondary combustion chamber, or a catalytic oxidizer such as an afterburner. Thermal desorption may use either direct/indirect heat exchange or air/inert gas to transfer vaporized contaminants from the contaminated medium.

Thermal desorption has been widely applied to treat tar-contaminated soils, refinery waste, wood-treating waste, creosote-contaminated soils, hydrocarbon-contaminated soils, nonhalogenated VOCs, SVOCs, PAHs, PCBs, pesticides, mixed (radioactive and hazardous) waste, synthetic rubber processing waste, and paint waste. The bed temperatures (from 170 to 550° C) and residence times used by thermal desorption systems will volatilize selected contaminants and drive off water, but typically will not oxidize or degrade organic compounds. Thermal desorption followed by direct combustion (e.g. using an afterburner) can be linked to incineration, and has the potential of having acceptability problems with local communities if used to treat hazardous waste.

There are different thermal desorption units available, including direct fired (e.g. natural gas) rotary units, indirect fired, hot oil rotary screw units, molten metal (e.g. tin) , bath units and infra-red heated batch units. Although thermal desorption units are commonly available, some systems may not be appropriate for treating chlorinated waste streams (CMPS&F, 1997).

Pyrolysis

Pyrolysis is an established *ex-situ* remediation technology. It is a technique of chemical decomposition where the hazardous organic compounds are transformed, under pressure and heat, into gaseous components such as methane, carbon monoxide, hydrogen and a residue of ash and carbon contents. The technology is useful in the treatment of pesticides contained in oily sludge, sediments and soils. This technology is usually linked to a pre-treatment technology, such as thermal desorption or soil vapour extraction. Further discussion regarding pyrolysis is provided in section 3.2.2.

3.1.3 Biological technologies

Table 6. Main limitations of established biological technologies

	Bioventing	Composting/ biopiles	Land farming
Technical/economical	Soil heterogeneity and low permeabilities may reduce efficiency. Low moisture content can limit biodegradation.	Large area is needed. Existence of metals may affect the clean-up performance. The final volume increases due to amendment addition (for composting). Medium- to long-term time to reach clean-up levels.	Chlorinated and nitrated compounds may affect pollutants degradability. Climatic conditions may increase time required to clean up. Not suitable for PCBs.
Social	No public opposition.	No public opposition (with proper odors and emissions control).	No public opposition.
Environmental	Potential fugitive emissions of by-products or hazardous compounds. Requires off-gases trapping systems.	The risk of fugitive emissions may limit the treatment of pesticides. Requires odour control and off-gases trapping systems.	Potential release of VOCs or hazardous compounds during tilling.

Biological techniques use micro-organisms or enzymes to degrade chemical contaminants. The key factor for bioremediation is to identify and/or develop the appropriate bacteria/fungi and a thorough understanding of how they survive, reproduce and grow in optimal conditions. Parameters such as temperature, humidity, pH available oxygen, substrate, soil/waste properties and degradation metabolites, must be controlled and understood in order to obtain effective results.

The USEPA has chosen bioremediation as a primary reasonable remedy to treat organic contaminants (including some POPs) in soils, sludge, and sediments at wood-treating sites (USCOTA, 1995). Bioremediation technologies were selected as treatment techniques for 17 of 47 sites in the US Office of Technology Assessment wood site remediation technology survey.

Bioventing

This method uses air supplied through injection wells and, in some cases, circulated by vacuum extraction. The airflow increases the volatilization of organic contaminants while simultaneously creating a proper environment for the biodegradation of the less volatile organics. Although there are many variants of this technology, the basic principle is to deliver low and optimized airflow rates that provide enough oxygen to the zone of contamination and 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, while vapours move slowly through biologically active soil and minimize volatilization of contaminants (USAEC, 1999). There are different methods used to supply oxygen to the subsurface, including vertical and horizontal bioventing wells; in some cases bioventing is combined with SVE (soil vapour extraction) to increase the control and flow of the injected air.

The method is applicable for 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 using this technique. Bioventing is not appropriate for the treatment of metal and inorganic contaminants.

Land farming

Land farming is a well-known remediation technology for the treatment of petroleum hydrocarbons contaminated soils. It is a technique designed to enhance the microbial degradation of contaminants by periodic tilling to induce aeration, controlled moisture content and addition of nutrients such as nitrogen and phosphorus. Pope and Matthews (1993) proposed a relatively standard methodology for this technology. The contaminated soil is usually excavated onto a designed lined bed (to avoid leaching) and mixed with a controlled amount of nutrients and soil additives such as bulking agents. Bioaugmentation of microbial culture can also be performed to enhance the degradation rate.

The treatment is appropriate for small quantities of pesticides that can be diluted and applied to land at controlled rates. Some pesticides are biodegradable, decomposing through the action of naturally occurring micro-organisms in soil. Land treatment is appropriate for pesticides that are susceptible to biodegradation in a short period of time (less than 26 weeks), under either aerobic or anaerobic conditions. The soil micro-organisms' activity can be enhanced through the addition of biologically active materials such as compost, sewage sludge, or night soil.

Composting

Composting is an *ex-situ* solid-phase remediation technology. Unlike land farming, this technique requires thermophilic (55 to 65° C) conditions due to the increased biological activity in the degraded organic waste. The contaminated soil is excavated and mixed with bulking agents and organic additives (such as wood chips and vegetative waste) to improve soil texture for aeration and drainage. Proper additive selection ensures adequate porosity and provides a balance of carbon, nitrogen and phosphorous to promote thermophilic microbial activity. The system is optimized by controlling (via irrigation) moisture content, pH, temperature and nutrients (Bossert et al. 1995), as well as the optimal carbon-to-nitrogen ratio. At the end of the process, an organic-rich compost remains; this material can then be placed back onto the contaminated site, providing a fertile soil for reforestation.

The composting process is applicable to soils contaminated with biodegradable organic compounds, heavy oils, PAHs, and munitions (explosives) waste 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 and 96.8 per cent, respectively (FRTR, 1997).

Biopiles

Biopiles or "engineered biopiles" are a modification of the land farming method for petroleum hydrocarbons decontamination, and advantageous because of the relatively small area needed, as well as the capturing and treating 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 in order to prevent further contamination and includes a delivery aeration system. In addition, it features an irrigation/nutrient system applied to the treatment heap and a leachate collection system used to recycle the collected fluid. Moisture, heat, nutrients, oxygen, and pH are controlled parameters to enhance biodegradation of the contaminants. This process normally reduces the contaminants to carbon dioxide and water within three to six months of operation.

For PCBs the process requires the anaerobic dechlorination in a first stage fermenter, where the chlorinated congeners are reduced to less than three chlorines per biphenyl molecule. Afterwards an aerobic stage is used to degrade PCB congeners that contain three or fewer chlorines per biphenyl molecule (Freeman, 1997).

3.2 Emerging and innovative technologies

3.2.1 Physico-chemical technologies

Base catalyzed dechlorination (BCD)

The base catalyzed dechlorination (BCD) system was developed to treat halogenated organic compounds. It is claimed that BCD is applicable for treatment of waste that contains up to 100,000 mg/kg of halogenated aliphatic or aromatic organic compounds such as PCBs. The formation of salt within the treated mixture may limit the concentration of halogenated material able to be treated. Rogers (1991) reports a reduction of chlorinated organics to less than 2 mg/kg. The BCD process can involve direct dehalogenation or can be linked to a pretreatment method such as thermal desorption that yields a relatively small quantity of a condensed volatile phase for separate treatment by the BCD process.

The BCD technology involves the addition of an alkali or alkaline earth metal to the polluted material that contains one or more halogenated or non-halogenated organic contaminant compounds. The BCD patent states that the alkaline chemical can be added to the contaminated medium in an aqueous solution, or in a high boiling point solvent. When the solid chemical is added as a suspension in water, the water helps distribute the metal compound homogeneously throughout the contaminated medium.

A compound able to donor hydrogen is added to the mixture in order to provide hydrogen ions that react with the contaminants; this applies only when hydrogen ions are not already present in the contaminated material. The hydrogen donor compound may include the high boiling point solvent in which the alkali or alkaline earth metal compound is added, or it may include aliphatic alcohols or hydrocarbons, amines or other similar compounds. A source of carbon (such as sucrose) must be added to activate these compounds in order to produce hydrogen ions.

The mixture is heated and maintained long enough to totally dehydrate the medium. After the water is removed from the medium during the dehydration step, the alkali is concentrated to a reactive state. The medium is further heated at temperatures from 200 to 400° C with enough time (from 0.5 to 2 hours) to produce a reductive decomposition of the pollutants.

Table 7a. Main limitations of emerging/innovative physico-chemical technologies

	Base catalyzed dechlorination (BCD)	**Electro-chemical oxidation**	**Solvent extraction chemical dehalogenation/ radiolytic degradation**
Technical/ economical	Not economical to treat large volumes of aqueous waste. The waste may require pre-dilution to achieve required destruction efficiencies. Overall efficiency is limited by thermal desorption efficiency. Energy costs to treat pesticides waste may be higher, due to the solvents distilled from the mixture.	Highly dependent on soil moisture content. Requires neutralization of treated soil.	Less effective when treating weight organic and hydrophilic compounds. Requires secondary treatment (including extracted metals). Soil types and moisture may impact efficiency.
Social	Generally not regarded adversely by community.	No public opposition.	No public opposition.
Environmental	Potential to form dioxins and furans is low, since the system operates under an inert atmosphere and the process should dechlorinate dioxins. Exclusion of air is required to prevent auto-ignition of hot oil. Alkaline pretreatment and solvent extraction imply fire and explosion risks.	Acids handling implies spill risk.	Solvent extraction implies fire and explosion risks. Must be assured the proper handling, recycling and disposal of used solvents.

The mixture is neutralized by the addition of an acid. Depending on the nature of the feed material, the added substances and the site use, it may be possible for the treated material to be returned to the site, although land use limitations may exist if the material is oily and/or has a high salt content. The BCD process has the capacity to reduce PCB from 10,000 mg/kg to below detectable limits in approximately 2 hours (Rogers, 1991).

The BCD process mainly involves chlorine stripping; when treating chlorinated aromatic hydrocarbons, the removal of chlorine atoms causes an increased concentration of lower chlorinated species. This is not a problem for PCBs, but with components such as dioxins the lower congeners (e.g. TCDD) can be more toxic than the highly chlorinated congeners (e.g. OCDD); therefore the process must be monitored to ensure that the reaction continues to completion.

The BCD system is not appropriate for treating large volumes of aqueous media (including wet sludge) because of the cost to evaporate water. The technology is applicable for low volatility organic liquids and high volatility organic liquids.

Electrochemical oxidation

Electrochemical oxidation was initially developed for the high-efficiency conversion of several radioactive organic wastes into environmentally acceptable waste streams. In tests with chemical warfare agents, this process, also called mediated electrochemical oxidation (MEO), was successful in destroying an organophosphorous nerve agent to non-detectable levels after one hour and an organochlorine agent (mustard) after two hours (CMPS&F, 1997).

The system includes an electrochemical cell used to generate oxidizing compounds at the anode in an acid solution (typically nitric acid). These oxidizers and the acid attack any organic compounds, converting most of them to carbon dioxide, water and inorganic ions at low temperature (< 80°C) and atmospheric pressure. The organic content of the feed, which may be soluble or insoluble organic liquids or solids, can vary from 5 to 100 per cent without affecting the process. In the same manner, the water content of the waste can vary over a wide range. Some compounds destroyed by this process include aliphatic and aromatic hydrocarbons, phenols,

organophosphorous and organosulphur compounds, chlorinated aliphatic and aromatic compounds.

Solvent extraction—chemical dehalogenation—radiolytic degradation

This *ex-situ* physiochemical reduces the volume of the pollutant that needs to be destroyed. The technology uses an extracting chemical to dissolve target contaminants from soils in a final solution for treatment with recovery of the solvent used. This process produces relatively clean soil or sediment that can be returned to the original site or disposed on 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 waste and heavy metals.

Solvent extraction technology can be applied to soils contaminated with volatile and semi-volatile organic compounds and other higher boiling point complex organics, such as polynuclear aromatic hydrocarbons (PAHs), petroleum hydrocarbons, pesticide/insecticide, polychlorinated biphenyls (PCBs), dioxins, and pentachlorophenol (PCP). Recent USEPA regulatory guidelines allow soil extraction with non-harmful solvents for removal of PCBs (USEPA, 1998).

Solvent extraction techniques are cost-effective methods to treat PCBs and other chlorinated compounds, but the main limitation is that the contaminants transferred to another phase must be destroyed through a secondary method. Different approaches have been developed to combine solvent extraction with other techniques, such as chemical dehalogenation with immobilized reagents (CDP) and gamma-ray irradiation. Recent studies show that the PCB concentration in transformer oil was reduced from 700 ppm to non-detectable levels in less than five minutes using chemical dehalogenation. While the results for radiolytic degradation showed that the PCB concentrations decreased with an increased γ-ray dose, nearly 60 mega rads were needed to degrade PCBs from 300 ppm down to 1 ppm in solvent saturated soil (Nam et al., 1999).

Solvated electron

This technology uses sodium metal dissolved in liquid anhydrous ammonia to produce a dark blue solution; the solvated electrons act as dehalogenating agents. Solvated electron solutions are rapidly formed when alkali or alkaline earth metals are dissolved in ammonia or in some amines, forming solutions containing the metal cation and free electrons.

Halogens can be separate from organic halides to yield a fully substituted parent hydrocarbon and a metal halide. The treatment of waste with "solvent electron" is performed with low to medium temperatures and the conversion of the waste occurs in seconds. It has been stated that there is no need for pretreatment, although some dewatering of sludge and/or sediments might be required.

It is claimed the technology can be used to treat halogenated hydrocarbons, pesticides, dioxins, PCBs, herbicides, CFCs, and chemical warfare agents. It is also stated that wastes have been successfully treated in bulk pure material, soils, sludge, sediments, porous and non-porous surfaces, oils, contaminated vessels, hardware and contaminated clothing.

The process design employs a cement-mixer-like reactor in which contaminated material and liquid ammonia are mixed. The ammonia completely disperses the soil, including the clays, and washes the contaminant from the soil. After brief mixing, a reactive metal charge is added (commonly calcium). The electrons released from the calcium rapidly dehalogenate the contaminants. Ammonia is recovered for further use, and the soil is deodorized. The decontaminated soil is suitable for return to the site, suitable for agricultural use since it is enriched in nitrogen from trace amounts of residual ammonia. The method is able to treat soils with up to 25 per cent water content. Supercritical water oxidation

Supercritical water oxidation

Supercritical water oxidation (SCWO) is an *ex-situ*, high temperature and pressure technology that uses the properties of supercritical water to destroy organic compounds and toxic waste. Under supercritical conditions, carbon is converted to carbon dioxide and hydrogen to water; chlorine atoms derived from chlorinated organic compounds to chloride ions; nitro-compounds to nitrates; sulphur to sulphates; and phosphorous to phosphate.

The properties of supercritical water are used to operate this process. Gases such as oxygen and organic substances are completely soluble in supercritical water, whereas inorganic salts present reduced solubility under supercritical conditions. Organic substances dissolve in the super critical water, and oxygen and the organic substances are brought into intimate single phase contact at temperatures and molecular densities that allow the conventional oxidation reactions to carry out rapidly to completion.

Process residues are contained if the waste contains inorganic salts or organics with halogens, sulphur or phosphorus. The effluent gases contain no oxides of nitrogen or acid gases such as hydrogen chloride or sulphur oxide. The process does not generate particulates and less than 10 ppm carbon monoxide has been measured.

It has been stressed that this system must be constructed of materials capable of resisting corrosion caused by halogen ions. The precipitation of salts may cause plugging problems in the system (Thomason, 1990). Destruction and removal efficiencies of greater than 99 per cent have been reported for the treatment of numerous hazardous organic compounds. SCWO can be

Table 7b. Main limitations of emerging/innovative physico-chemical technologies

	Solvated electron	Supercritical water oxidation	Solar detoxification—photochemical degradation
Technical/ economical	May require a pretreatment for dewatering of sludge and/or sediments.	The end products (ash and brine) require proper disposal. Limited to treat liquid waste with solids size of less than 200 μm. Applicable to waste with organic content less than 20%.	The photolysis rates for pesticides are highly dependent on latitude, season and other meteorological conditions.
Social	No public opposition known at this stage.	No known public opposition at this stage.	No known public opposition.
Environmental	Ammonia is a volatile liquid; toxic and fire risks. Calcium metal combined with hydrogen may form explosive mixtures.	Due to the high temperatures and pressures used in this technology, requires specialized control equipment, reactor materials and safety practices.	Low environmental impact due to limited use of chemicals and low off-gas generation rates.

applied to aqueous waste streams, sludge and contaminated soils. It is also applicable to treat acrylonitrile wastewater, cyanide wastewater, pesticide wastewater, PCBs, halogenated aliphatics and aromatics, and organic nitrogen compounds.

Solar detoxification—photochemical degradation

Solar energy can be used to degrade organic compounds of synthetic and natural origin. Short wavelengths (295–400 nm) of the solar spectrum are greatly attenuated by the atmosphere, so that radiation is able to generate direct and indirect photolytic processes that can degrade pesticides and PCBs polluting soil and surface waters. Since the mentioned wavelengths are attenuated more strongly than longer visible wavelengths, the rate of photolysis of pesticides is highly dependent on latitude, season and other meteorological conditions; thus, in tropical regions photochemical processes are a key factor to assess pesticides fate and degradation (Plimmer, 1998).

Solar energy is used to degrade hazardous organic chemicals by direct thermal decomposition or by photochemical reaction. Some advantages include savings in fuel use, improved thermal destruction of contaminants, and a reduction in exhaust gas volumes, including PICs (products of incomplete combustion). These processes can use either thermal energy or a range of photochemical reactions.

In order to efficiently use solar energy it is necessary to concentrate the solar radiation to achieve the high temperatures to decompose or destroy contaminants. Solar radiation is reflected by mirrors (heliostats) and absorbed by a receiver reaching temperatures of up to 2,300° K. No auxiliary fuel is required and it has been demonstrated to show an improvement in the destruction and removal efficiency (DRE) of organics, including pesticides, by a factor of 100 or more against conventional thermal technologies. High destruction efficiencies can be achieved at a temperature of 750° C, which is lower than the temperature required for thermal incineration.

The main photochemical processes that aid thermal treatment in solar detoxification include photocatalytic oxidation using titanium dioxide (TiO_2) as a catalyst. Ultraviolet radiation is used to promote an oxidation reaction in photocatalytic reactions using a catalyst such as TiO_2 in the presence of oxygen. The reactivity of singlet oxygen, irradiated with visible light in the presence of dissolved oxygen, is used in the dye-sensitizer processes. The reactive species produced can then react with contaminant molecules in the waste.

Oxidative degradation of pesticides, including Lindane in contaminated water has been tested with direct sunlight in a solar furnace. Singlet oxygen was effective against some of the pesticides but reacted slowly or not at all with others. All pesticides were degraded by OH radical generating agents (such as methylene blue). Each system has different capabilities, which needs to be taken into consideration when making comparisons (Funken, 1997).

Gas phase chemical reduction

Gas phase chemical reduction (also known as eco logic process) has been developed as an alternative to incineration technologies. The technology is based on gas-phase thermo-chemical reaction of hydrogen with organic compounds. Hydrogen combines with organic compounds at 850° C or higher, in a reductive reaction to form lighter hydrocarbons (mainly methane). For chlorinated organic compounds, such as PCBs, the products are methane and hydrogen chloride. The reaction is carried out with water, which functions as a reducing

agent and generating hydrogen. The technology is a hydrogenation process and adds hydrogen atoms to any incompletely hydrogenated organic molecule, dechlorinating molecules and breaking down aromatic rings, therefore is non-selective in its treatment of organic substances.

The process can quantitatively convert PCBs, PAHs, chlorophenols, dioxins, chlorobenzenes, pesticides and herbicides, to methane. The yield will be determined by the concentration of organics in the waste. Approximately 40 per cent of the methane produced can be further converted to hydrogen through the water shift reaction and non-reacted methane is converted to hydrogen in the catalytic steam reformer. The process can therefore operate with the hydrogen produced itself.

The gas phase reduction process is likely to be preceded by a thermal desorption unit when treating solid waste. There is potential for the removal of organic contaminants from the solid material to be improved in the chemical reduction process, as the thermal desorber will operate under a reducing hydrogen atmosphere, offering simultaneous destruction. The technology needs water in its operation and therefore can process waste with relatively high water content. This aspect provides an advantage over other thermally based processes that require treatment for sludge with high water content.

Catalytic hydrogenation

The destruction of halogenated waste by hydrogenation in presence of noble metal catalysts has been studied for many years. Although, noble metal catalysts are particularly susceptible to poisoning by several substances found on waste, thus limiting the applicability of the technology. A process has been developed for the regeneration of PCB contaminated transformer fluids using hydrogenation catalysts based on metal sulphides, which are extremely robust and tolerant to most catalyst poisons (Musoke, 1982). The process is also claimed to destroy a wide range of chlorinated hydrocarbons, forming hydrogen chloride and light hydrocarbons as by-products.

In different trials relatively high concentrations of pure POPs compounds were treated in a hydrocarbon solvent and all were destructed to levels below the detection limit of analysis, presenting destruction efficiencies from 99.9996 per cent (for hexachlorobenzene) to 99.99999 per cent (for 1,2,3,4-TCDD). It is claimed that the variations in destruction efficiencies reflect the differences in the instrument detection limits rather than real differences in the extent of destruction (Duffy et al., 1997). Most off-gases are recycled through the reactor, although purge gases are discharged through a catalytic combustion chamber.

Different surveys have shown that successful dechlorination of polychlorinated aromatic compounds by using Ni catalysts requires severe reaction conditions, high temperature and high hydrogen pressure. Pd, Ru catalysts that permit successful dechlorination of polychlorinated aromatic compounds under mild conditions are not developed for large-scale applications because of their high cost (Hagh, 1990).

Recent surveys have shown the preparation of a selective catalyst to convert environmentally problematic compounds into useful products, allowing the performance of liquid phase hydrodechlorination under mild conditions, using bi-metallic catalysts consisting of nickel or copper associated with palladium, supported on a high-surface area carbon. The results show that such bi-metallic systems permit the carrying out of liquid phase hydrodechlorination of hexachlorobenzene under mild conditions (P_{H2} 1 atm; T = 50° C), and that the method of catalysts preparation has a strong effect on their selectivity (Simagina et al., 1999).

3.2.2 Thermal technologies

Thermal desorption integrated technologies

The technologies involving thermal desorption as a pretreatment-separation technique integrated with a post treatment-destruction technology are presented at this point.

Thermal desorption—catalyzed dehalogenation

This system is composed by a thermal desorption system linked to the base catalyzed dechlorination (BCD) system. The system uses an indirectly heated thermal desorber to split organic compounds from contaminated media (Sheih, 1994). The system is designed to achieve feed material temperatures of up to 510° C, allowing an effective treatment of soils and sludge polluted with a wide range of low and high boiling point compounds. The system is applicable for hydrocarbons, pesticides, herbicides, PCBs, coal by-products, wood treating compounds, dioxins and furans. The gases produced during the process are treated by a vapour recovery system which includes an oil venturi, an oil scrubber, a water scrubber, a condensing unit and vapour phase carbon adsorption unit.

Contaminants and moisture volatilized from the contaminated material are entrained in the off-gas and are condensed and recovered by the scrubbers/condensers. The condensed mixture is separated and the organic contaminant is collected for recycling via solvent recovery, fuel substitution or treatment using the BCD process. Separated water can be treated by liquid phase carbon adsorption and sand filtration. Most of the treated water can be recycled back to the process for use in the scrubbers and cooling conveyor.

Thermal desorption—pyrolysis

The PCS (*product control soméus*) technology is based on thermal desorption combined with flash pyrolysis technique, and followed by combustion. The main operational units of the system include an indirectly heated rotary reactor, indirectly cooled solid material cooler, and multi-venturi scrubber, pyrolysis gas combustion chamber, water treatment, auxiliary equipment and automatic operation with continuous monitoring.

Table 7c. Main limitations of emerging/innovative physico-chemical technologies

	Gas phase chemical reduction	Catalytic hydrogenation
Technical/economical	Pollutants such as sulphur and arsenic may inhibit treatment. Sulphur in combination with iron may produce slimes that require additional centrifuge separation. The existence of irregular solids may also limit waste treatment due to materials handling. May need to be linked to special waste handling facilities in order to improve waste material handling.	Potential poisoning of catalysts may decrease or nullify process efficiency.
Social	Generally not regarded adversely by community.	No public opposition.
Environmental	Potential fugitive emissions of PCBs, pesticides or dioxins. The handling, use and storage of hydrogen within the process represent fire and explosion risks. The facilities must be subjected to an internal hazardous operations review and specialized process control to prevent release of waste materials during a process upset.	Gaseous products may generate safety and toxicity hazards. Combustion products may require scrubbing that would generate aqueous waste.

Table 8. Main limitations of emerging/innovative thermal technologies

	Thermal desorption integrated technologies	Plasma arc systems	Vitrification
Technical/ economical	Overall efficiencies of methods are limited by thermal desorption efficiency, which depends on soil type and conditions.	The removal of volatile metals and particulates formed from inorganic components may require treatment; these additional steps may increase the cost. This process usually has a relatively high capital and operating cost. Some systems are limited to treat liquids and gases. Solids can only be treated after extraction or by forming slurry mixtures.	Vitrification is a destructive process and the soil can no longer be used for agricultural purposes. The vitrified matrix may hinder future use of the site if done *in-situ*.
Social	In some cases may attract public opposition.	Generally not regarded adversely by community.	No known public opposition.
Environmental	Combustion of off-gases requires control and emissions treatment. Process conditions must be selected and controlled in order to minimize the risk of dioxin and furan formation, and require pollution control equipment to treat these in the event that small quantities are formed.	The absence of combustion gases results in a gas emission smaller than for incineration systems. A surge tank is provided to contain any uncontrolled release of gases from the treatment chamber. The use of mechanical seals and operation of the unit at slight negative pressures should prevent any fugitive emissions.	Cautions must be taken to prevent fugitive emissions of vaporized organics. The vitrified nature of the formed matrix greatly reduces any potential leaching of metals or other residual pollutants.

The rotary reactor is the main component of the system. Waste is partially vaporized in a reductive environment under low vacuum conditions (0 to 50 Pa). The reactor is cylindrical in shape, arranged horizontally and rotates around its axis. The operating temperature in the reactor ranges from 450° to 800° C. The waste may be introduced directly, or after drying in a desorber. If needed, the waste is ground in a mill in order to homogenize to a size less than 5 mm. The waste is decomposed into solid and vapour phases, which include heavy metals in water insoluble form, high boiling point organics in the solid phase, and volatile organic compounds, volatile heavy metals and halogens in the vapour phase.

After pyrolysis, the vapour phase is combusted and rapidly cooled; the gas stream is cleaned in a wet gas scrubber prior to emission. Although dioxin and furan gases are not generally formed in a reductive environment, it is possible that they could be formed following the combustion step. Therefore, after combustion, the resulting gases must be treated by scrubbing. The scrubber process water is cleaned, neutralized and water recirculated.

The process applications include the conversion to energy of waste such as solid hazardous waste, PCB contaminated soil, mercury contaminated soil, hospital waste, municipal solid waste, sewage sludge and coal. Besides, the technology can treat a full range of chlorinated hydrocarbons, organochlorine pesticides, all organic and/or inorganic materials with combined contamination of organics, halogens and heavy metals. Although this technology is not applicable for treatment of liquids (water, flammable liquids and solvents), explosives and/or materials with highly oxidizing nature under heat treatment and materials that cannot be decomposed by thermal treatment at 600° C.

Thermal desorption—retort system

This technology is adapted to treat contaminated soils containing volatile organic compounds (VOCs) or some semi-VOCs. The process has been configured for the treatment of pesticide contaminated soils, especially for dip sites.

The system involves an indirectly fired retort that is used to remove the volatile materials through an off gas-vent, leaving the treated soil for return to its original site. The retort operates on a continuous basis under negative pressure, and under neutral conditions (i.e. neither oxidizing, nor reducing) resulting in some leakage of air into the system. The treated soil leaves the retort via an overflow washer from where it is transferred to a stockpile.

The retort contents are indirectly heated. A combustion chamber surrounds the retort and the components are initially brought up to operating temperature by heating a batch charge of inert material. When this mass is at opening temperature, feed is started. Bed temperatures are monitored to ensure that conditions are maintained by varying either the feed rate or the firing rate; temperatures are set in the range of 400-700° C depending on the residence time required, type of contaminant and soil properties. Typically, in treating organochloride pesticide contaminated soils the retort operates with a bed temperature of 450° to 500° C (CMPS&F, 1997).

Within the retort the pollutants are volatilized and/or decomposed and separate as part of the off-gas. The off-gases are then drawn by a fan through a hot gas filtration system that removes particulate matter, allowing the cleaned gases to go to an afterburner for the residual organics destruction. The afterburner is designed to operate at 1,100° C with a two-second-residence time. From the afterburner, the gases are quenched to minimize dioxin and/or furan formation.

Retort process is only able to treat solids and sludge, although liquids (e.g. pesticides formulations) could be treated by first producing a slurry. Treatment of low volatility compounds such as PCBs is not proposed on the current development status.

Plasma ARC systems

This technology uses high temperatures (around 10,000° C) for pyrolysis, which result 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 compound by the superheated cloud of gas or plasma into atomic elements and subsequent treatment converts the atomic forms into innocuous substances.

A thermal plasma field is created by directing an electric current through a low-pressure gas stream. Plasma arc ranges can reach 5,000° to 15,000° C. There are different variations of plasma arc processes like PACT (plasma arc centrifugal treatment) (USEPA, 1992), PLASCON (in-flight plasma arc system) and STARTECH (plasma-electric waste converter) (CMPS&F, 1997).

Vitrification

The soil is treated with high temperature to cause a melt and form a glass when cooled. This technology can either be carried out *in situ* or *ex situ*; consists of inserting graphite electrodes into the contaminated encased area and energizing with a high electrical resistance heating (more than 1,700° C) to melt soil into a molten block. It is applicable for the treatment of organics (including pesticides and PCBs), inorganics and radionuclides. The organic contaminants will normally be destroyed while the inorganics will be trapped into the vitrified matrix. The plasma arc centrifugal treatment (PACT) mentioned above is a combination of plasma arc and vitrification techniques (CMPS&F, 1997).

3.2.3 Biological technologies

Biological techniques are commonly carried out with indigenous micro-organisms since these present superior performance due to the better survival rates compared to strains taken from geographically different locations (non-indigenous inoculants). However, some studies have illustrated that the use of indigenous micro-organisms for bioremediation and as hosts for developing genetically engineered organisms does not provide any advantage in dynamic and highly competitive environments. Thus, the survey recommends that the site must be engineered to provide temporal advantages for the non-indigenous micro-organisms, or the known inoculant must be able to degrade a specific site better than the native strain (Blumenroth and Wagner-Dobler, 1998).

Bioslurry

This is a proper technique for sites that require greater process control, more complete and faster degradation rates. The contaminated soils are mixed with water to form a slurry in order to allow contact between micro-organisms and contaminants. The slurry is then fed into a bioreactor where a controlled amount of air is supplied for mixing and aerating; inoculation may be performed to enhance treatment. If conditions (temperature, nutrient concentration and proper aeration) are optimized, slurry processes are faster than other biological processes. The treated slurry is suitable for direct land application, similar to composted soils (Freeman, 1997).

The clean-up time is less than twelve months. Slurry-phase bioreactors are used to remediate soils and sludge contaminated with explosives, petroleum hydrocarbons, petrochemicals, solvents, pesticides and other organic chemicals. Bioslurry is favoured over *in situ* biological techniques for heterogeneous soils, low permeability soils and areas where underlying groundwater is difficult to capture.

Enhanced bioremediation

Enhanced bioremediation, also called biostimulation or bioaugmentation, is a process to increase 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 (Freeman, 1997). Although it could be done in anaerobic conditions, it is more advantageous when oxygen is not limiting in order to prevent the formation of persistent by-products such as vinyl chloride resulting from the anaerobic degradation of trichloroethylene.

This technique can be used *in situ* to treat soils contaminated with different pollutants such as petroleum hydrocarbons, solvents, pesticides, wood preservatives and/or nitrotoluenes.

Table 9. Main limitations of emerging/innovative biological technologies

	Bioslurry	Enhanced bioremediation
Technical/ economical	Dewatering soil fines after treatment can be expensive. An acceptable method for disposing of non-recycled wastewater is required.	Water-based solution circulation may move pollutants to underlying groundwater. Clogging may occur. It is not suitable for low permeability soil. High metal and chlorinated organic concentrations can be toxic to the organisms. This technology is not effective at low temperatures.
Social	Not regarded adversely by community.	Not regarded adversely by community.
Environmental	Caution and operational conditions must be set to prevent potential fugitive emissions of pesticides.	Some POPs may be formed under anaerobic conditions. The mobilization of contaminants may affect the surrounding environment (air and groundwater).

4. Recommendations and conclusions

The difference between technologies that only separate and/or concentrate a pollutant (e.g. solvent extraction, thermal desorption) and those which destroy the contaminant (e.g. pyrolysis, oxidation, reduction, biodegradation) must be considered when setting site remediation goals. Those technologies that only immobilize contaminants (e.g. landfill cap systems, stabilization and vitrification) should also be clearly differentiated.

To select the most proper technology several ratable and non-ratable criteria should be considered. Among "non-ratable", or relative criteria, are included public acceptability, risk and environmental impacts, which depend on the specific geographic site location. The ratable criteria may include the applicability of the method (in accordance with its development status), overall cost, minimum achievable concentration, clean-up time required, reliability, maintenance, post treatment cost and ability to use soil after treatment. Social, environmental, technical and economical criteria should be considered during the technology selection process. The more criteria involved, the better are the results obtained. In case of choosing more than one technology to treat a specific waste or soil, the limitations, impacts and risks due to the combined methods should be considered.

The applicability and availability of the different treatment technologies depends on the location of treatment systems and whether the waste can be transported to the treatment facility. Pesticides or PCBs are transported between countries, depending on the availability of treatment systems within the country in which the waste is generated, as well as the quantities involved. While it is desirable to minimize the transport of pesticides and PCBs, the transport and mobilization of these contaminants will continue until enough movable units or *in-situ* treatment systems are available within the countries that generate the waste.

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B. NEWS AND EVENTS

UN and other organizations' news

UN talks on GM trade protocol collapse

United Nations talks to establish an international protocol on the trade and use of genetically modified organisms (GMOs) in Cartagena, Colombia, were suspended, to the relief of the European biotechnology industry.

The European biotechnology industry association, EuropaBio, said the biosafety protocol was so flawed that "it is better to have no protocol", because it would have meant "more red tape and bureaucracy". The main opposition from industry groups was against including commodities such as soya and oilseed rape.

Paul Muys of EuropaBio said industry would welcome a protocol that "proved we are serious about maintaining bio-diversity".

Officials met to discuss the risks posed to biodiversity and human health by biotechnology, and the implications for developing countries. Talks stalled over the scope of the proposed treaty's powers, liability for environmental damage from GMOs, and minimising the impact on trade. While many countries have domestic legislation on GMOs, there is currently no international agreement on trade or accidental GMO release across national borders.

A lobby led by the US blocked a proposed compromise, which would have allowed restrictions on imports of experimental organisms and crops. EU environment commissioner, Ritt Bjerregaard, said the negotiations should be relaunched and concluded, with or without the US.

The talks are to resume within 16 months, although further details have yet to be decided.

Meanwhile, Germany's biotechnology industry association, the DIB, said it still sees a chance to pass a biosafety protocol.

The DIB stated the Advanced Informed Agreement process for transport of live modified organisms (LMOs) should apply only to the first import and that the protocol should apply only to LMOs that could threaten biodiversity. (Source: *European Chemical News*, 8-14 March 1999)

UN to end children's vaccine initiative

The Children's Vaccine Initiative (CVI)—an alliance of United Nations agencies, private foundations, and industry set up in 1990 to improve vaccination programmes for the poorest children in the world—is being disbanded after eight troubled years. No announcement about its future has yet been made, but it will be replaced later this year with a new structure for promoting cooperation between public and private sector groups in the international vaccine community. The details have not yet been worked out.

The vaccine industry will be sad to see the demise of the CVI, because it gave companies a strong voice with the UN agencies in policy and planning. But others seem to have few regrets. The alliance, observers say, was often hamstrung by turf battles between agencies such as the World Health Organization (WHO) and the UN Children's Fund (UNICEF).

CVI is supported by a grant of $2.5 million per year, principally from WHO, UNICEF, and the World Bank. It was established in 1990 with the aim of reducing the number of children dying from preventable infectious diseases. Its remit was to set priorities for global vaccine development and delivery, promote collaboration between agencies, and find new sources of money.

Despite the high hopes for the initiative, it failed to raise significant amounts of new money or to coordinate the vaccine community fully.

For the past year, the global vaccine community has been discussing how to improve its record of immunizing the world's poorest children. Finally, at a meeting in Bellagio, Italy, senior officials from industry and the UN agencies recommended that each agency strengthen its own internal efforts to collaborate and that the CVI should become a scaled-down operation with a coordinating role but no responsibility for policy, fund-raising, or setting priorities.

Industry is hoping CVI will be replaced by an independent body in which it would have equal status with the agencies. An announcement is expected in September or October. (Extracted from *Science*, vol. 283, 26 March 1999)

UN to move on gene resolution

The United Nations (UN) is nearing approval of a resolution calling for restrictions on human gene research and respect for genetic diversity.

A UN committee approved the Resolution on the Human Genome and Human Rights, which calls for vigilance against discrimination based on a person's genes and recommends restrictions on human cloning and germline gene therapies, which risk introducing new genes into a population. The resolution also argues that use of human DNA "should not give rise to financial gain"—a controversial issue as companies race for lucrative gene patents.

Observers say the panel's endorsement virtually ensures that the measure will pass a General Assembly vote. But whether nations will adhere to the guidelines is uncertain. Germany and Australia, which are still working on their own policies, have expressed reservations. And the United States pressed to soften the guidelines before endorsing them. Georgetown University bioethicist LeRoy Walters says Americans generally have "less hesitancy" than others about genetic manipulations. (Source: *Science*, vol. 282, 27 November 1998)

FAO supports organic farmers worldwide

Consumer demand for organically produced food is on the rise and provides new market opportunities for farmers and businesses around the world, according to a new report from the UN Food and Agriculture Organization (FAO). Typically, organic exports from developing countries are sold at impressive premiums, often at prices 20 per cent higher than identical products produced on conventional farms. The report states that, under the right circumstances, market returns from organic agriculture can potentially contribute to local food security by increasing family incomes, and recommends an FAO-wide, cross-sectoral programme in organic agriculture.

In several developed countries organic agriculture already represents a significant portion of the food system: 10 per cent in Austria and 7.8 per cent in Switzerland. Other countries such as the US, France, Japan and Singapore are experiencing growth rates in the organic industry that exceed 20 per cent annually.

Some developing countries such as Egypt have small domestic organic markets and have begun to seize the lucrative export opportunities presented by organic agriculture, FAO said. Some countries export tropical fruits to the European baby-food industry, six African nations export cotton to the European Community, Zimbabwe exports herbs to South Africa, and China exports tea to the Netherlands and soybeans to Japan.

Entering the market in industrialized countries is not easy for organic producers in developing countries, according to FAO. In most cases, farmers seeking to sell products in developed countries must hire an organic certification organization to inspect their farms annually. These services can sometimes be expensive, and few developing countries have certification organizations within their borders, according to the report.

FAO recommended that it would be advantageous for farmers to participate in locally-based, applied field research. Experience with FAO-initiated Integrated Pest Management (IPM) Farmer Field Schools and community forestry projects has shown that farmers can practice good scientific methods if they are given training and technical support.

To maintain consumer confidence in the integrity of organic products, FAO recommends that countries promote their own organic certification organizations and better enforce organic standards by "punishing those who engage in fraudulent activities as well as undertaking systematic tracking and measuring of fraud and its impact on the market".

The report concludes by stating: "FAO has the responsibility to give organic agriculture a legitimate place within sustainable agriculture programmes and assist member countries in their efforts to respond to farmer and consumer demand in this sector. Organic agriculture may contribute to the overall goals of sustainability."

The FAO report, *Organic Agriculture* is available on the web at www.fao.org/unfao/bodies/COAG/COAFG15/default.htm.

Sources: UN FAO Press Release 99/3, "Organic Agriculture", Item 8 of the Provisional Agenda, Committee on Agriculture, 25-29 January 1999.

Contact: UN FAO, Viale delle Terme di Caracalla, 00100 Rome, Italy; tel.: (39-06) 5705 3105; e-mail: Erwin.Northoff@FAO.org (Source: *Global Pesticide Campaigner*, vol. 9, No. 1, April 1999)

Back on the menu

The biotechnology industry has emerged largely unscathed from recent discussions at United Nations (UN), European Union (EU) and UK level, designed to weigh up the benefits and risks of genetically modified organisms (GMOs).

At the world agriculture conference in Rome, the UN's Food and Agriculture Organization (FAO) spoke of the benefits of biotechnology in feeding the world's growing population, reducing agrochemical use and making plants tolerant to conditions in marginal areas, but called for adequate biosafety regulations and risk assessments to avoid harmful effects.

The European Parliament gave a vote of confidence to the industry in its review of proposed amendments to the European Union's directive on the "deliberate release" of GMOs. Despite widely divided opinions on the subject, the Parliament's environment committee voted that time-limits should not be incorporated into new GMO product approvals, in contrast to the European Commission's proposal for approvals to be reviewed after seven years.

In the UK, a House of Lords parliamentary report supported GM crops and called for faster EU procedures for product approvals. However, the report was attacked by the Government's own nature advisory committee,

English Nature. Separately, 120 British and Irish food writers signed a pledge not to use GM food and called on the Government to ban it. (Source: *European Chemical News*, 1-7 February 1999)

New maize genetics research centre

A new maize genetics research centre at the University of Missouri-Columbia "will expand scientists' capacity to improve corn as a food and feed crop through harnessing biotechnology and computers to crack the plant's genetic code", asserted Eileen T. Kennedy, USDA Deputy Under Secretary for Research, Education, and Economics. US Department of Agriculture scientists and university collaborators announced the establishment of the Maize Genetics Research Center in mid-January 1998. The centre is being funded through a five-year, $11.1 million grant from the National Science Foundation.

The maize project includes collaboration among scientists at USDA's Agricultural Research Service in Columbia, Mo. And the University of Missouri. Others participating in the plant genome project are from Clemson University, South Carolina, and the University of Georgia. (Source: *Diversity*, vol. 14, Nos. 3 & 4, 1998)

HUGO consolidates offices, web sites

The Human Genome Organisation (HUGO), whose purpose is to promote international collaboration within the Human Genome Project, has merged its HUGO Americas office with the London entity. The London web site lists regional HUGO contacts and links to the HUGO Pacific office, publications and reports, and information on HGM '99 and other genome meetings (www.gene.ucl.ac.uk/hugo; e-mail: hugo@hugo-international.org; Pacific office: web, hugo-pacific. genome.ad.jp; e-mail, tito@ims.u-tokyo.ac.jp or shobu@ims.utokyo.ac.jp). (Source: *Human Genome News 10 (1-2)*, February 1999)

Gates launches $100 million initiative

The planet's richest individual is donating a portion of his fortune in hopes of buying some of the world's poorest children a priceless gift—good health. Bill Gates, the chair of Microsoft Corp., gave $100 million to create the Bill and Melinda Gates Children's Vaccine Program. The programme will enlist existing international health organizations in a battle against four diseases through its support of vaccine trials, public education, and new funding mechanisms. "Our goal is to make the vaccines you and I take for granted available to children no matter where they live", Gates said at a press briefing in New York City.

The donation comes from the William H. Gates Foundation and will be administered by a Seattle-based organization called the Program for Appropriate Technology and Health (PATH). The money, to be given over 10 years, will fund efforts to improve delivery of existing vaccines rather than to develop new ones, says Gordon Perkin, president of PATH. In particular, it is aimed at disseminating vaccines proven effective against:

- *Haemophilus influenzae* type b (Hib), which causes pneumonia and meningitis;
- Rotavirus, which causes severe diarrhea and dehydration;
- Hepatitus B, which causes cirrhosis and liver cancer; and
- *Streptococcus pneumoniae*, which causes ear infection and pneumonia.

About three-fourths of the money is expected to go to the World Health Organization (WHO), the United Nations Children's Fund (UNICEF), and the International Vaccine Institute (IVI). The fledgling IVI, based in Seoul, Korea, has already received $250,000 to supplement drug company funding of a study of the distribution of Hib throughout China, Korea, and Viet Nam.

PATH has assembled an international advisory panel of seven eminent scientists that will meet in March to recommend ground rules for the new programme. But two funding priorities are clear: to coordinate cost-effectiveness studies and trials to improve the vaccines' performance in the developing world, and to explore new ways of financing large-scale childhood immunization efforts, such as interest-free loans from the World Bank.

The Gates programme will not pay for the tens of millions of doses that will be needed throughout the world, says Perkin. Even so, says Carol Bellamy, executive director of UNICEF, the donation is certainly welcome. (Source: *Science*, vol. 282, 11 December 1998)

A unique alliance to map genetic variability

The era of personalized medicine, when patients will be prescribed drugs tailored to their precise genetic make-up, has come a step closer.

Ten major drugs companies and the Wellcome Trust, the world's largest medical charity, are unveiling a plan to map the variability of the human genome within two years. The data will immediately be made public to head off attempts by other companies to patent the information for their own gain.

The $45-million project will identify and analyse single nucleotide polymorphisms (SNPs). These are variations in single DNA bases, which account for most of the genetic differences between people. SNPs are thought to be a major determinant of people's susceptibility to disease and their response to drugs.

The ongoing Human Genome Project, which aims to have a working draft of our entire genetic blueprint by February 2000, will produce a "consensus" sequence for the typical human. But David Bentley, head of genetics at the Wellcome Trust's Sanger Centre near Cambridge, argues that individual variation is even more important. Up to a tenth of the three million SNPs thought to exist will be mapped by the new consortium.

The Wellcome Trust is providing $14 million for the project. The rest will be made up from equal contributions by the 10 companies: AstraZeneca, Bayer, Bristol-Myers Squibb, Hoffman-La Roche, Glaxo Wellcome, Hoechst

Marion Roussel, Novartis, Pfizer, Searle and SmithKline Beecham.

An original proposal for an SNP map from the British pharmaceuticals giant Glaxo Wellcome evolved into a consortium when the companies agreed that they would make more rapid progress by first collaborating on identifying SNPs and then competing on efforts to make drugs using the information.

The Sanger Centre, the Washington University School of Medicine in St. Louis and the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, will be responsible for identifying SNPs. Those that look as if they might be medically important will then be mapped by the Human Genome Center at Stanford University in California and the Sanger Centre. Finally, the information will be analysed and built into an overall genome map by the Cold Spring Harbor Laboratory on Long Island, New York.

By making the information freely available, the consortium hopes to prevent other companies from patenting key SNPs. But it could find itself in a race against firms that have decided to go it alone. Genset, based in Paris and San Diego, says it intends to build its own SNP database and file patents. Celera Genomics of Rockville, Maryland, is also investing heavily in SNPs.

However, the consortium has already made a start. The Sanger Centre has completed a three-month pilot project which mapped SNPs on chromosome 22. (Source: *New Scientist*, 17 April 1999)

Green groups sue to stop BT crops

A coalition of environmental groups and organic farmers has filed a suit to force the US Environment Protection Agency (EPA) to cancel registrations for genetically engineered crops containing *Bacillus thuringiensis* (*Bt*) insect toxins. The coalition, led by Greenpeace and the Center for Food Safety (CFS; Washington), sued after EPA rejected a petition they had filed in September 1997.

The coalition says a management plan crafted by *Bt* crop developers and under consideration by EPA will not block insect tolerance to the *Bt* toxin upon which organic farmers rely. "EPA has shown a blatant disregard for federal law and its own regulations by approving *Bt* crops without fully assessing their environmental safety", says CFS legal director Joseph Mendelson. (Source: *Chemical Week*, 24 February 1999)

Regulatory issues

GM ingredients fuel concerns by consumers

Public anxiety in Europe about the safety of genetically modified plants, which has triggered a backlash against GM products in the food sector, is beginning to impact the cosmetics and pharmaceuticals industries as well.

European supermarket chains are declaring that their own-label food products will be free of genetically modified organisms (GMO). In March 1999, seven supermarket companies in the UK, France, Switzerland, Belgium, Ireland and Italy formed a consortium to buy GM-free crops and derivatives worldwide.

Even some fast-food companies are banning GM ingredients from their menus in response to public concerns about what the media have dubbed "Frankenstein" crops.

Now a similar anti-GM stance is being taken by cosmetics outlets. The biggest of these is The Body Shop International, which has 1,600 stores in 48 countries and also manufactures its own-label products.

The UK-based company, which specializes in products with natural ingredients, says it will buy oils, such as those from soya and corn, from only certified GM-free sources. It warns that it will switch from existing suppliers to producers able to give assurances about non-GM oils.

The company is calling for a five-year moratorium on the commercial growth and sale of GM crops in Europe while further research is carried out into their possible effects on human health and the environment.

Most GM crops are currently grown in North and South America because few licenses for GM commercial crops have been issued in Europe.

The European Cosmetics industry says it may soon have to ask suppliers about the origin of their ingredients.

Pharmaceutical manufacturers are also worried that, because of public pressure, European legislation requiring food labels to provide data about GM ingredients could be extended to medicinal products, particularly vitamins and other dietary supplements.

The Proprietary Association of Great Britain (PAGB), a trade association for manufacturers of over-the-counter drugs, is warning its members to prepare for legislative changes.

The cosmetics industry notes that only a tiny fraction of the ingredients in its products come from GM crops.

"Probably less than 1 per cent of over 9,000 ingredients used in the industry may have been derived from GMOs", says Rory Macmillan, communications manager at the European Cosmetic Toiletry and Perfumery Association (Colipa). Those ingredients, known as genetically modified derived ingredients (GMDIs), usually come from GM plants that have passed stringent safety regulations, he adds.

Nevertheless, the industry may have to work with suppliers to identify GM-sourced materials.

The EU has a regulation, mainly relating to Novartis' *Bacillus thuringiensis* (*Bt*) maize and Monsanto's soybeans that are resistant to its Roundup herbicide, stating that any foods containing detectable GM proteins or DNA must be labelled.

But food manufacturers and companies in other sectors say the regulation needs clarification. For example, it does not specify what test methods should be used.

The European Commission, the EU executive, is developing a list of products like refined vegetable oils and starch hydrolysates that would have undetectable GM proteins or DNA even if derived from GM crops. Such products would be exempted from labelling rules.

A range of companies and organizations say there should be a system of crop segregation under which GM and non-GM produce would be separated in the distribution chain.

Iceland, a UK supermarket chain, has appealed directly to the American Soya Bean Association, which represents US soya bean growers, to introduce crop segregation.

The chain is obtaining non-GM soya from Brazil and Canada. It guarantees that after 1 May, none of its own-label food products will contain GM ingredients. (Source: *Chemical Market Reporter*, 29 March 1999)

How to price what we put on our plate

For most people, the main question about GM food is: do I have to eat it or not? If we are to have that choice, GM crops will have to be segregated from plough to plate and all products containing GM food labelled as such.

The US Government claims that this would impose heavy costs on its food suppliers. It threatens a trade war if the European Union responds to public pressure by demanding segregation of GM crops within US exports. But a new analysis suggests that the costs of segregation and labelling are manageable, and could even enhance trade. "This could be the only key to easing public acceptance of biotechnology", says Allan Buckwell, an agricultural economist at Wye College near Ashford, Kent.

Buckwell presented his findings in Brussels earlier this month. He says that similarly stringent segregation—although not on the basis of genetic modification—is already widespread. "Different varieties of wheat, for bread or pasta, are already strictly separated from farm gate to production plant", he says. And in the US, soya growers already distinguish beans used in different kinds of tofu for export to Japan.

The cost of such segregation is not prohibitive, say Buckwell and his colleague Graham Brookes. For example, soya growers and processors in the US separate and label beans with different protein and oil contents for an extra cost of just 6 to 9 per cent compared with unsegregated beans. Soya growers in Brazil distinguish GM from non-modified soya for a premium of 10 to 15 per cent. European dealers separate maize with a high oil content for 17 per cent extra cost, while US producers do it for 6 per cent. Canadian farmers distinguish GM from normal oilseed rape, or canola, for an 8 per cent premium. And costs will come down, says Buckwell, if segregation becomes widespread. (Source: *New Scientist*, 27 February 1999)

Crop engineers on the defensive

Health scares in Europe, such as HIV-contaminated blood supplies, are the reason from the region's hostility toward genetic engineering, says biotech industry group EuropaBio (Brussels). The group argues that there is no scientific evidence that plant biotechnology is harmful and calls such concern "pure speculation".

However, independent research published over the past year provides evidence that the industry is overstating its case. Studies identifying environmental and health risks from specific genetically modified crop traits, as well as risks inherent in genetic engineering, cast doubt on assumptions supporting the industry's broad safety claims.

Health concerns flared when toxicologists confirmed a study in which genetically engineered potatoes fed to rats damaged their immune systems and organs. The study had been dismissed by industry groups after officials said they found "inconsistencies" in the data. The potatoes were modified with a gene that codes for a toxic lectin protein that defends beans and other plants against herbivores. The study shows that crop modifications can carry health risks from one plant to another. Pioneer Hi-Bred reported similar findings in studies where nut proteins that were transferred to other plants caused reactions in people allergic to nuts.

The possibility of accidental trait transfer dominates environmental research on genetically modified crops. A study by Dutch researchers in January heightened concerns that antibiotic resistance genes used to develop transgenic crops could be passed to bacteria in the human gut. Researchers from the Dutch State Institute for Quality Control of Agricultural Products (Wageningen) and the TNO Nutrition and Food Research Institute (Zeist) studied the fate of DNA in an artificial gut. The simulation predicted that DNA survives undigested in the gut for several minutes—long enough for bacteria to pick it up.

Environmental research has demonstrated that the possibility of genes passing from crops to wild plants—so-called outcrossing—may also be greater than expected. University of Chicago ecologist Joy Bergelson reported last fall that transgenic mustard crops are 20 times more likely to interbreed with wild relatives than are mustard crops modified through traditional breeding.

The ecological threat from *Bacillus thuringiensus* (*Bt*) insect toxins in transgenic crops may also be greater than previously thought. New York University biologist Guenther Stotzky found that unlike the toxins used in *Bt* sprays, which rapidly biodegrade in soil, *Bt* toxins from transgenic crops can accumulate and threaten desirable soil insects.

Regulators, grower groups, and crop developers in the US and Canada are introducing codes of farming practice for *Bt* crops. But these codes are designed to limit development of resistance to *Bt* by insects, not to eliminate health and environmental risks. European regulators are moving with greater caution: The UK Government has delayed the planting of herbicide-tolerant crops until spring 2000 while it assesses ongoing trials, and French authorities have banned planting of transgenic crops other than corn pending studies on outcrossing.

Opposition from farm groups in developing countries and antibiotech activists may block a technological fix to cross-breeding: genes that render transgenic crops sterile. Agbiotech companies are developing these "terminator" genes to prevent farmers from using seeds from a crop for future plantings. Critics of the technology fear the growing control of multinationals such as Monsanto, DuPont, and Novartis over seed supplies.

Crop developers and some scientists insist that plant biotech is safe and say the regulatory system is working.

Even researchers whose work has been trumpeted by activists point out that their studies do not definitively prove a risk. For example, the Dutch artificial-gut researchers say their study demonstrates only the possibility of antibiotic resistance genes spreading to bacteria in the gut. But many scientists say the studies show the need for greater scrutiny of transgenic crops by regulators—especially with the broadening use of the technology. (Source: *Chemical Week*, 3 March 1999)

Ethical issues

Britain urged to expand embryo studies

Biologists in Britain who want to use human stem cells to develop new medical therapies say the chances for government support are looking brighter. They are encouraged by an opinion issued in London by a senior advisory panel urging the UK Government to enact a new law to ban "reproductive cloning" of humans while permitting a limited type of cloning for research on new methods of treating disease.

The recommendations, written by a joint working group of the two agencies that regulate the use of human reproductive technology in the country—the Human Genetics Advisory Commission (HGAC) and the Human Fertilisation and Embryology Authority (HFEA)—are expected to carry substantial weight in the UK. The report could also become a model for other countries, say US researchers.

The joint HGAC and HFEA working group began reviewing UK policy last January at a time when the press was full of speculation that humans might soon be cloned. The working group drew up a summary of key issues and sought public comment. On cloning for reproductive purposes, the outcome was "conclusive", "86 per cent of the people who commented supported a ban on human reproductive cloning". The working group also endorsed a total ban.

But a fraction of respondents also favoured limited research that involves DNA transfer into oocytes, the process that produced the sheep Dolly. Besides offering a way to copy an organism, cloning might enable researchers to transfer DNA from a defective to a healthy embryo, and it might also allow them to create new tissue for transplants. The working group supports research in these two areas.

In the first, aimed at studying diseases rooted in the mitochondria—the cells' energy-producing organelles—DNA might be transferred from a cell with deficient mitochondria into a healthy oocyte, creating an embryo that could develop into a healthy child. The goal of the second line of research would be to clone a patient's DNA in stem cells derived from an embryo and coax those cells to develop into tissues that would be accepted by the patient's immune system. The potential medical value, it adds, is "enormous". The report recommends that research licenses be granted for these areas of research.

Existing UK guidelines allow researchers to obtain a license for research on human embryos up to the 14th day of development, but only for narrow applications such as improving fertilization methods. Under the proposed new rules, however, these early embryos could be used for broader purposes, such as developing stem cells that can grow into a full range of specialized tissues.

The HGAC-HFEA report lets agencies know that they should now give serious consideration to grant requests in these areas.

The decision on whether to accept these recommendations, however, rests with the health ministry and the HFEA. (Source: *Science*, vol. 282, 18 December 1998)

Tissue report pits privacy against pathology

Efforts to protect genetic privacy could hold up cancer research in the US, pathologists claim. They are worried about a report from the National Bioethics Advisory Commission (NBAC) on the use of human tissue samples held in repositories throughout the country.

Many of the samples were gathered during surgery, and most are decades old. Advances in DNA technology mean that researchers can now extract a wealth of information from them. The potential benefits to medicine are enormous.

But there are risks to privacy. Decades after someone underwent surgery, for example, a researcher might discover that they carry a disease gene which—if the information leaked out—could threaten the person's ability to obtain insurance.

The draft report says that nearly all proposals to use tissue samples in research should be reviewed by local institutional review boards, which already vet research on human volunteers. The boards would rule whether sample donors must be contacted to obtain their consent. (Source: *New Scientist*, 16 January 1999)

Weighing in on bioethics

Cloning, assisted suicide, managed health care—these and other ethical hot-button issues show no sign of fading from public debate. To get up to speed, check out The Bioethics Internet Project. "Ours is the most visited of the primary bioethics sites", claims project director Glenn McGee of the University of Pennsylvania Center for Bioethics.

One innovative item on the 5-year-old site is a joint project with the NBC television drama *ER*. Each week, a centre master's student gets an advance copy of the upcoming episode and dissects it in an online essay. The

site also has links to scholarly journals, including Penn's own *American Journal of Bioethics*. A section on assisted suicide presents legal documents from a recent US Supreme Court case, an online roundtable discussion, and related web links. An especially popular feature is "Bioethics for Beginners", which offers a historical introduction to medical bioethics and a list of papers and web sites for students and teachers. According to McGee, "Genetic engineering is now the topic for high school debate classes".

The Bioethics Project does not stake out positions on issues, says McGee, but centre faculty members do post their own strong viewpoints. "Some bioethics sites are funded by large companies", he notes, "but the centre is independent. In bioethics, conflict of interest is everything". (Source: *Science*, vol. 284, 23 April 1999)

Biosafety

Panel to improve protections for people in drug experiments

A panel of experts at the University of Pennsylvania's Center for Bioethics headed by Art Caplan recommends updating protections for human subjects in drug research.

New oversight is needed, the scientists said, due to changes in the research environment over the past two decades.

The regulations, developed 20 years ago, were designed to deal with clinical trials conducted largely by the NIH or academic institutions with one investigator and at one site.

The recommendations, published in the December issue of the *Journal of the American Medical Association*, also address the multi-centre character of clinical trials, "which creates unique problems for IRBs [institutional review boards], the informed consent process, and the monitoring of research activity", the report states.

A result of four years study, the recommendations focus on developing increased protections for those participating in clinical trials and greater accountability for researchers and institutions.

In particular, it urges special provisions for certain populations, including cognitively impaired persons—citing new research in Alzheimer's and Parkinson's diseases—be added to the federal regulations, last updated in a major way in 1981.

The report urges that consent forms include clear language that discourages unrealistic expectations in research; that human subject protections be extended to cover research which is not federally funded and not FDA-regulated; that more stringent rules be issued concerning the 1997 emergency research provision which allows experimental treatments on patients in emergency situations without informed consent; and that individuals in unequal power relationships such as students, not be required to participate in research without being given reasonable alternatives to participation.

Moreover, the report urges that local review be strengthened and continued, and to facilitate this, that local IRBS are given more responsibility and accountability.

"Remarkably little is known about what actually transpires in the course of research, partly because IRBs do not currently have the practical ability to invest more of their resources in monitoring riskier studies", the report states.

It also urges that IRBs be informed of potential conflicts of interest on the parts of investigators, including "finder's fees" given to physicians who recruit patients, as well as those with financial ties to drug companies. It also urges that the Office for Protection from Research Risks (OPRR) be separated from the NIH because of potential conflicts of interest.

Finally, the committee urges that the National Bioethics Advisory Committee consider whether some form of national review be required for particularly sensitive research, such as foetal tissue research or germline therapy.

"In the final analysis, science is a social enterprise", it states. "Like all science, biomedical and behavioural research with human subjects can only fulfil its promise if it is worthy of the wider society's trust."

The NBAC is due to publish its findings on the status of human research in the next months. (Source: *McGraw Hill's Biotechnology Newswatch*, 21 December 1998)

Negotiations on biosafety protocol derailed

An international biosafety protocol is being negotiated under the auspices of the Convention on Biological Diversity (CBD) to regulate the safe transfer, handle, and use of living modified organisms (LMOs). Officials from 148 governments attended the Sixth Meeting of the Open-ended Ad Hoc Working Group on Biosafety on 14 to 19 February 1999 which was followed by the Extraordinary meeting of the Conference of the Parties (ExCOP) on 22-23 February 1999 in Cartagena, Colombia. The Working Group concluded its work but was unable to present a consensus text for adoption by the ExCOP. Whilst most of the articles of the draft protocol have already been decided as acceptable to all parties, disagreements could not be settled on main issues of the protocol such as trade issues, treatment of LMOs sold in bulk as commodities, such as genetically modified maize and soybean, and the discrepancy between domestic vis-à-vis international regulation.

During the negotiations there emerged three main coalitions which formed the negotiating groups: first, the "Miami Group" comprising of Argentina, Australia, Canada, Chile, United States and Uruguay; second, the "Like Minded Group" comprising of mainly the developing countries of the Group 77 (exclusive of those that were members of the Miami Group), China and the Central and Eastern European countries; third, the "Compromise Group" comprising of Japan, Mexico, Norway, the Republic of Korea, Switzerland and the

European Union (EU). In the final analysis, the Miami Group could not accept a compromise on the draft Protocol which had been proposed by the EU and agreed on by all the other states.

Amongst the most contentious issues was article 5, which outlines the advance-informed agreement (AIA) procedure. This procedure is at the core of the protocol because it requires the exporter of LMOs to inform the country of import prior to the first intentional transboundary movements of these LMOs. Many developing countries are of the opinion that all LMOs should be subject to the AIA procedures as any LMO, irrespective of intended use, could be accidentally released during transfer and handling. However, the current draft protocol creates an exemption by providing that the intentional introduction of LMOs into the environment does not include LMOs "intended for direct use as food or feed, or for processing". The Miami Group argued that therefore all commodities containing LMOs should be excluded from the AIA procedures of the protocol. Instead, it was proposed to allow countries to perform risk assessment and approval for these commodities under their domestic law.

Another reason for disagreement among governments was article 31 of the protocol, covering trade protective provisions. In this article it is stated that "the provisions of this protocol shall not affect the rights and obligations of any party to this protocol deriving from any existing international agreement to which it is also a party", unless this "would cause serious damage or threat to biological diversity". The Miami Group has proposed to delete this last phrase of the article. The EU has proposed to delete the entire article and place it in the preamble. Many representatives from developing countries also argued for its deletion because it mirrors CBD article 22. The Miami Group had concerns about the potential conflict of a biosafety protocol with the treaties of the World Trade Organization (WTO) on free trade. According to Rafe Pomerance, the head US negotiator, this is a crucial issue since the USA was not willing to accept that this protocol will undermine the WTO trading regime.

To continue on the formulation of a biosafety protocol all participants agreed that as a first step informal consultations would be held in September 1999. The ExCOP will be resumed at a later date prior to the next ordinary meeting of the COP, which will be held in May 2000.

Volker Lehmann, Editor, Biotechnology and Development Monitor.

Sources: Miller, H. I. (1999), "Cynicism and Politics Dominate UN Biotechnology Deliberations". *Nature Biotechnology*, vol. 17, No. 6, p. 515.

http://www.biodiv.org/excop1/html/engl/excop1-2.htm

http://www.iisd.ca.linkages/vol09/enb09117e.html

Personal communications with T. Yongo (Secretariat of the CBD). (Source: *Biotechnology and Development Monitor*, No. 38, June 1999)

General

Investment crisis

The US biotechnology industry is facing a funding crisis while on the verge of major breakthroughs, a new report claims.

Accountancy firm Ernst & Young says in its 13th annual review of the biotech industry that investors are increasingly unwilling to invest in biotechnology companies. But they say this is "not entirely illogical" given the high-risk nature of the business and the extreme volatility of biotech stocks.

The industry's return on investment has not been sufficient to reward investors for assuming a high level of risk, the report notes. Whether this is merely a temporary downturn or a long-term change in investor attitudes remains to be seen, it says.

Biotech firms, however, can increasingly rely on "life-sustaining" injections of cash from alliances with pharmaceutical giants. "This 'marriage of convenience' is likely to continue, with the pharmaceutical industry gaining access to technologies to provide the next blockbuster drug" the report says.

Despite the lack of investor confidence, the report claims that biotechnology will be a leading industry in the next century thanks to the accelerating pace of discovery and the healthcare demands of an ageing population. (Source: *Chemistry & Industry*, 15 March 1999)

Coalition sues US EPA over Bt crops

A coalition of environmentalists, organic farmers and consumer groups has filed a lawsuit against the US Environmental Protection Agency (EPA) to force the agency to end its approval of genetically engineered *Bt* crops. The lawsuit was filed by Greenpeace International, the Center for Food Safety, the International Federation of Organic Agricultural Movements (IFOAM), Pesticide Action Network North America and over 70 other plaintiffs including 34 farmers from 18 states. The suit charges EPA with wanton destruction of *Bacillus thuringiensis* or *Bt*, one of the world's most important biological pesticides, and calls on EPA to cancel registration of all genetically engineered *Bt* plants, stop approval of any new *Bt* plants and immediately perform an environmental impact assessment.

Bt is a naturally occurring soil bacterium used as a biological pesticide that can be cloned and inserted into a crop plant. The plant then produces the toxin in most if not all parts of the plant. It is highly likely that insects will develop resistance to *Bt* much more quickly when it is genetically inserted into the plant than when *Bt* is simply sprayed. The coalition argues that widespread cultivation of crops expressing *Bt* toxins threatens continued effectiveness of *Bt* sprays widely used by organic farmers. (Source: *Global Pesticide Campaigner*, vol. 9, No. 1, April 1999)

HIV epidemic grows

Each year, just before World AIDS Day on 1 December, United Nations AIDS officials release the latest statistics on the epidemic. This year's figures brought more bad news: An estimated 5.8 million people worldwide were newly infected with HIV in 1998, bringing the total number of HIV-infected people to 33.4 million. Over the same period, some 2.5 million people died of AIDS. Nearly 70 per cent of the new infections occurred in sub-Saharan Africa, which continues to be the hardest hit region of the globe. In several African countries, more than one-fifth of the adult population is already HIV-positive, while in others—most notably South Africa—the epidemic is growing so explosively that this figure will probably soon be reached.

"The worst is yet to come", predicted Agathe Lawson, the Côte d'Ivoire-based representative of UNAIDS—the UN's special AIDS programme—at a press conference last week in Paris, one of several venues where UNAIDS officials unveiled the depressing global figures. Yet, despite these extraordinary numbers, AIDS activists and physicians continue to question whether political leaders are treating the epidemic with the urgency it deserves. In South Africa, this simmering issue has boiled over into a major public controversy. South African health officials have decided not to provide the antiviral drug AZT to HIV-infected pregnant women—despite its proven effectiveness in preventing transmission of the virus to their offspring—because, they argue, it is too expensive.

Although Africa is currently taking the brunt of the epidemic, the new statistics show that no corner of the world will be spared the ravages of AIDS. Of particular concern is the growing HIV infection toll in India, a nation of nearly 1 billion people, where random sampling in rural areas has shown adult HIV infection prevalences reaching 2 per cent, while among women who visited clinics for treatment of sexually transmitted diseases the figure is as high as 13.6 per cent. Even in Western Europe and North America, where death rates from AIDS have plummeted thanks to cocktails of antiviral therapies, the proportion of the population infected with HIV is continuing to rise, with 74,000 new infections on the two continents during 1998.

Nowhere, however, is the situation worse than in sub-Saharan Africa, where more than a dozen countries now harbour adult HIV infection prevalences of 10 per cent or higher. In four countries—Botswana, Namibia, Swaziland, and Zimbabwe—more than 20 per cent of adults are now infected. (Source: *Science*, vol. 282, 4 December 1998)

Global teams to battle infectious diseases

Biomedical scientists in North America, the United Kingdom, and tropical nations will need to work together to win funding from a new $25 million research effort to fight infectious diseases.

The UK-based Welcome Trust and the US-based Burroughs Wellcome Fund unveiled an Infectious Diseases Initiative that aims to promote equal research partnerships among developed and tropical developing nations. "It is clear that forming global partnerships ... is a key step towards reducing the health toll of infectious diseases", said fund President Enriqueta Bond.

The multinational teams—which must include members from the United States or Canada, Britain, and a tropical nation—will compete for 5-year awards worth up to $4 million. The first proposals are due in January, with a decision expected in August. A second funding round is planned for 2000. (Source: *Science*, vol. 282, 30 October 1998)

HIV/AIDS pandemic is worsening

By Brian Halweil

Since the beginning of the AIDS epidemic in the early 1980s, the number of people infected with HIV—the virus that causes AIDS—has climbed to nearly 50 million. Nearly 6 million people were infected in 1998, and 2.5 million people died from AIDS. (Each year since 1980 there has been a record number of new infections and AIDS deaths.) Cumulative AIDS deaths stand at over 14 million, and with 34 million people currently living with HIV, the number of deaths is expected to keep climbing.

The region worst hit by the epidemic has been sub-Saharan Africa. Crippled by poverty and a lack of widespread prevention efforts, the region has been the site of 7 out of every 10 of the world's cases of HIV infection, and 9 out of every 10 deaths due to AIDS. In a dozen African nations, at least 10 per cent of the adult population now carries the virus. In the two hardest-hit nations, Zimbabwe and Botswana, one of every four adults is infected, and the average life expectancy has been cut by nearly 20 years.

In Asia, the rate of new infections remains relatively low, but the total number of people infected is expanding rapidly. The rate of infection is fairly low in India, for example, but with nearly 1 billion people, the country is home to an estimated 4 million infected individuals—more than any other nation.

Since 1994, the number of people living with HIV in Eastern Europe has surged nearly sevenfold. General collapse of economic and health care systems—on top of soaring drug use—has kindled the epidemic in the former Soviet bloc.

With the help of antiviral drugs that prolong the onset of AIDS, the number of AIDS deaths has declined in the United States and Western Europe, though new HIV infections are rising steadily as risky behaviours persist. In the United States, 64 per cent of new infections occur in blacks and Hispanics, who account for just 24 per cent of the population.

In contrast to other epidemics in human history, most of which predominantly affected the young and the elderly, AIDS has taken its most serious toll on working adults—the economically active cornerstone of a nation's development. As wage earners die off, families are forced to find alternate sources of income. In addition, the

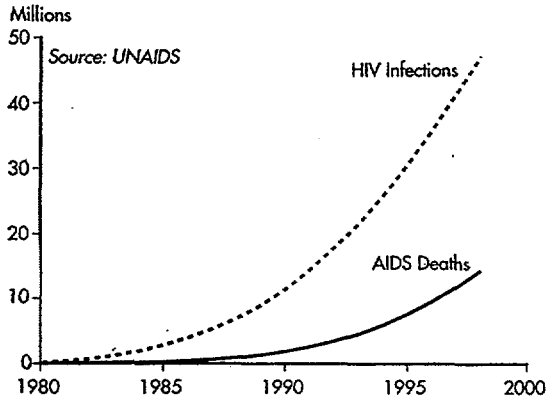
number of children orphaned by AIDS now exceeds 9 million, and that number is expected to grow substantially in the future.

National health-care systems in the countries hardest hit are being overwhelmed by the epidemic. The estimated cost of providing antiviral treatment to all infected individuals in Malawi, Mozambique, Uganda, and Tanzania greatly exceeds these countries' gross national products.

Impoverished peoples and nations are now faced with the brunt of the epidemic, according to participants at the 12th Annual World AIDS Conference in July 1998. Because of inadequate access to health care and education, the have-nots of the epidemic have been the least prepared to prevent the spread of AIDS. For example, in Latin America—as in other regions—infection rates are most severe in impoverished nations, such as Bolivia and Honduras.

At the same time, infection rates have slowed or declined in several nations that adopted strong prevention programmes, including Senegal, Tanzania, Thailand, and Uganda. Successful efforts have included free distribution of condoms, needle-exchange programmes, sex education at all levels of schooling, and support from religious and civil leaders.

In roughly half of the developing world, the epidemic still has not spread widely in the general population or even in high-risk groups. That provides a huge opportunity for governments who realize that prevention now costs a fraction of the price of treatment later. (Source: *World-Watch*, March/April 1999)



Estimates of cumulative HIV infections and AIDS deaths, worldwide, 1980-1998

C. COUNTRY NEWS

Australia

Australian Patent Office fee reductions

The Australian Patent Office has announced that it will abolish continuation and renewal fees for the third and fourth years for any case on which such a fee falls due on or after 1 November 1998. In addition, sealing fees for grant of a patent for which the sealing deadline is after 1 November 1998 have been abolished, and are refundable if they have already been paid. The fee for requesting examination has been reduced from AU$ 350.00 to AU$ 290.00 for any case in which the request for examination is lodged after 1 November 1998, provided that the Direction to Request Examination has been issued after 1 May 1998. (Source: *Australasian Biotechnology*, vol. 8, No. 6, December 1998)

Seed safeguards

The Australian Government has tightened its rules on patenting seeds after being accused of biopiracy by farmers from developing countries. Rural development groups criticized the Plant Breeders' Rights Office in Canberra for granting patents on more than a hundred varieties of seed from Asia, Africa and Latin America. Now agricultural researchers applying for patents in Australia will have to disclose the origin of their seeds and prove that they have bred different varieties. (Source: *New Scientist*, 21 November 1998)

Read the label

Health ministers in Australia and New Zealand decided in December 1998 that all genetically modified food sold in the two countries must be labelled as such from 1 May this year. The countries' food producers are annoyed that labelling will be required even if the genetic modification does not affect the food's taste, size or nutritional value, and claim that the ruling will reduce their international competitiveness. However, the European Union has already adopted similar rules, putting Europe and Australasia at odds with the US on the issue. The US argues that enforced labelling of modified foods creates unfair trade barriers. (Source: *New Scientist*, 9 January 1999)

New Centre on Metals and Genetics opens in Melbourne

The opening of the Centre for Cellular and Molecular Biology at Deakin University in February establishes a major international research centre for the study of the effect of heavy metals on human health.

Headed by Professor Julian Mercer, a world-renowned leader in the field of the biology of copper, the centre also paves the way for a new course in human genetics that will be offered by the Faculty of Science and Technology next year.

Before joining Deakin last year, Professor Mercer spent 18 years at the Murdoch Institute at Melbourne's Royal Children's Hospital where he led a team that isolated the gene involved in the fatal genetic copper disorder, Menkes disease. The discovery of this gene has revolutionized the study of the biology of copper, a metal which is essential for health and is associated with diseases of the brain including Alzheimer's disease, motor neurone disease and mad cow disease (bovine spongiform encephalopathy). His team has moved with him from the Murdoch Institute to Deakin and is continuing work on the project. Other staff members are studying the health effects of metals such as zinc, iron and arsenic. (Source: *Australasian Biotechnology*, vol. 9, No. 1, April 1999)

Austria

Austria may go it alone on GM

Austria may introduce mandatory consumer labelling for genetically modified food additives independently of the rest of the European Union, unless the EC acts, says Barbara Prammer, consumer affairs minister.

Prammer expressed concern that the Commission had not managed to issue its own proposal to expand labelling requirements from ingredients such as soya to additives.

Prammer said that she is "prepared to pass a national decree" to force companies to label food containing genetically modified materials by 3 September.

She also warned the biotechnology industry to maintain complete "transparency and honesty" over plans for field tests of GM crops in Austria. (Source: *European Chemical News*, 15-21 March 1999)

Maize planting abandoned

A coalition of biotechnology firms with interests in Austria has given into negative public opinion and cancelled the first national planned field trial of 100 m^2 of genetically modified (GM) maize.

A spokesperson for the "Forum Biotech" coalition—which comprises biotech companies such as Monsanto Pioneer and AgrEvo and food companies—told *European Chemical News* (ECN) they can legally begin trials but attitudes to GMOs are "too negative" to justify seeding this year.

Austrian environmental group Global 2000 called the move "a step in the right direction".

Although the Austrian Government recently reiterated its opposition to a moratorium on GM crop trials, Forum Biotech said the relevant government ministries have declined to cooperate with the Forum on a public education programme on the subject.

"Without their support, it would just look like propaganda", said the spokesperson. (Source: *European Chemical News*, 19-25 April 1999)

Canada

Milk hormone faces growing opposition

Canada has banned bovine somatotrophin (BST), a genetically engineered growth hormone that increases milk yields when injected into dairy cows.

A panel of veterinary experts appointed by the Canadian Government concluded that mastitis, an udder infection, is 25 per cent more likely to occur in cows treated with the hormone. Treated cows are also 18 per cent more likely to be infertile and 50 per cent more likely to become lame.

Monsanto of St. Louis, MI, which makes BST, claims the panel unfairly included data on experimental versions of the hormone made by other companies.

Since its launch on the US market in 1993, BST has been one of the biotech industry's most controversial products. The European Union has imposed a moratorium on its sale, arguing that its use would drive small farmers out of business. This expires in January 2000, but the Canadian ruling will increase pressure to impose a permanent ban. (Source: *New Scientist*, 23 January 1999)

China

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 P.R.C. 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. That office will work with Chinese officials in an effort to set up governmental seed performance testing and to establish relationships for marketing Pioneer products. The P.R.C. 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 & Product Development, 7300 NW 62nd Avenue, P.O. Box 1004, Johnston, IA 50131, USA. Tel.: +1-515-270-363; Fax: +1-515-253-2478; e-mail: <mcconelr@phibred.com> (Source: *Diversity*, vol. 14, No. 1 & 2, 1998)

Cuba

Cuba's billion-dollar biotech gamble

Although Cuba remains a poor nation, the country has begun to attract attention for a surprising reason: its huge investment in biotechnology. President Fidel Castro is staking much of his nation's science resources on a roller-coaster industry dominated in the United States, at least, by venture capitalists. The leader's devotion to biotech has raised eyebrows among industry and academic experts, but this grand capitalistic experiment shows tantalizing hints of succeeding. Several thousand scientists at Havana's biomedical campus and at satellite centres have already developed a couple of dozen products, including monoclonal antibodies, streptokinase—a drug used to break up blood clots—and the world's only available vaccine against meningitis B. Under development are cancer vaccines and other compounds that would be considered cutting-edge in US labs.

But Cuba's fledgling industry faces major obstacles to competing with its rivals in developed countries. The 38-year-long US embargo has isolated researchers from colleagues and pharmacy shelves in the United States, and Cuban biomedical institutes are only haltingly gaining the acumen needed to market products. Cuban biotech officials admit they have a long way to go to secure a place in the world market. "This is a new industry in Cuba", says Center for Genetic Engineering and Biotechnology director Manuel Limonta. "In many places in the world, biotechnology companies do not even have revenues".

Behind the biotech broom is Fidel Castro himself, who has made public health a priority—Cuba's infant mortality rate is the lowest in the developing world.

Despite losing a bid for a new United Nations biotech centre, Cuba in 1986 invested $120 million to build CIGB and launched several institutes nearby. The labs began training a cadre of scientists, most of whom won the coveted privilege of working abroad for a year or two.

At first the campus focused on products for use in Cuba, making preparations tested elsewhere, but after the Soviet Union collapsed in 1991, depriving the country of billions of dollars a year in subsidies from its patron, Cuba began selling its wares abroad, especially in Latin America. These products now include everything from a hepatitis B vaccine to immunoassays that require one-tenth as much reagent as standard plates, putting blood tests for neural tube defects, AIDS, and other conditions within reach of dozens of developing countries. Cuba earns about $100 million a year from such products—a drop in the bucket, perhaps, to most any US firm with a drug on the market. Nevertheless, says CIGB immunologist Jorge Gavilondo, the sales prove that "we have grown from a scientific institute to a biotech company".

Like any biotech company, CIGB and other Havana institutes pride themselves on their pipeline. Basic research is a growing part of CIGB's portfolio, says Limonta. CIGB and other institutes are working on vaccines against hepatitis C, dengue, and cholera, among other diseases. CIM, meanwhile, has pioneered cancer vaccines that trigger an autoimmune response to epidermal growth factor receptors, which are overexpressed in certain tumours, and to gangliosides found on tumour cell membranes. Nicholas Restifo of the US National Cancer Institute calls their ideas "really fresh and interesting".

Not everything the Cubans have touched has turned to gold. A much-touted initiative to develop an AIDS vaccine has faced the same stumbling blocks that bedevil similar efforts in other countries. Two years ago, CIGB gave 24 volunteers a cocktail of GP120 HIV coat proteins, which did trigger an immune reaction. The institute plans to carry out further trials next year. But most groups outside Cuba are now combining GP120 with other strategies that prime the body's cell-mediated immune response.

Still, observers say, the advances clearly are outpacing the setbacks—an amazing feat considering that the country, aside from its booming tourism industry, endures rationed food and gas, a dearth of basic medicines like aspirin, and minuscule wages. And, in spite of its privileged status, the biotech effort finds itself chronically short of funds in the wake of Cuba's economic crash a few years ago. Researchers scrounge for supplies and rely on foreign collaborators for access to pricey techniques such as X-ray crystallography.

Despite these problems, Cuba's biotech researchers enjoy plum conditions compared to scientists in other fields.

After the revolution?

For scientists who believe in Cuba's biotech dream and are determined to remain in the country, the elusive goal is breaking into markets in developed nations. But Cuba faces many obstacles, including the high costs of getting approval to sell products in such countries. Cuba is "weak" in quality-control standards and marketing skills and has only recently begun applying for patents, notes Mikael Jondal of the Karolinska Institute in Sweden, who two years ago served on a European fact-finding mission to Cuba. Cuban leaders respond that their labs now adhere to international standards for quality control and clinical trials. (Extracted from *Science*, vol. 282, 27 November 1998)

Ethiopia

Ethiopian workshop on TRIPS/CBD

Ethiopia's Institute for Sustainable Development and the Biological Society of Ethiopia organized a two-day workshop on 3-5 February 1999, to examine the conflict between Trade Related Intellectual Property Rights and the Convention of Biological Diversity. Some 140 participants agreed on several recommendations including: prohibiting patents on all life-forms, working towards the development of *sui generis* legal systems to protect the rights of local and indigenous communities to their genetic resources; integrating traditional and modern farming systems; raising awareness of the values and sustainable uses of biodiversity resources; and ensuring that extension and research approaches are area- and situation-specific.

For more information, contact: Sue Edwards, Director, Institute of Sustainable Development, Box 30231, Addis Ababa, Ethiopia. Tel.: (251-1) 204210; Fax: (251-1) 552350; e-mail: sustainet@hotmail.com/ sustain@telecom.net.et (Source: *Seedling*, March 1999)

European Union

Manufacturers remain optimistic despite the wait

Approval of nutraceuticals and genetically modified foods is being held up within the European Union because of concerns among member States about the safety of new food technologies.

Unilever has had to postpone the launch of a margarine containing a cholesterol-lowering sterol derived from soya oil. Zeneca is also likely to have to put back the introduction of a genetically modified tomato to consumers in continental Europe.

The two products and roughly 12 others are awaiting approval under a new regulation governing the licensing of novel foods throughout the EU. An evaluation dossier has been drawn up on all the products for study by the EU's 15 member States.

"A number of States have been asking for more information, while a few want new assessments and scientific evidence", says an official at the European Commission, the EU's executive. "It seems inevitable that a lot of

questions should be asked with a new approval process like this".

Once a dossier has been considered by the member States, it is then examined by an EU scientific advisory committee on foods and then by a standing committee of EU officials. "It could be several months before some of these products reach that final stage", the official notes.

The member States usually take only 60 days to examine a product, but the review of the Unilever margarine has already passed that deadline. (Source: *Chemical Marketing Reporter*, 15 March 1999)

European Biotechnology Directive passed

The long-awaited European Union Directive on the Legal Protection of Biotechnological Inventions has finally been passed by the European Parliament, and adopted by the Council of the European Union. The Directive was published on 30 July 1998 in the Bulletin of the European Union. Implementation of the Directive by member States of the European Union must be completed by 30 July 2000, and after that date national patent laws and Patent Office practice must comply with the directive.

The directive greatly clarifies and restricts the exclusions from patentability which have hitherto caused such difficulty for applicants for European patents because of the way in which they are currently expressed in the European patent convention. Patentability of isolated DNA fragments and of novel plants are not restricted to a specific plant variety is affirmed. "Essentially biological processes for the production of plants or animals" are defined restrictively, to consist "entirely of natural phenomena such as crossing of selection". (Source: *Australasian Biotechnology*, vol. 8, No. 6, December 1998)

EU Committee refuses first crop

The European Commission Scientific Committee on Plants (SCP) has rejected a potato developed by starch producer Avebe (Veendam, the Netherlands). It is the first transgenic crop SCP has decided not to recommend for Commission approval for sale and cultivation throughout the European Union (EU). Given rising concern in Europe about transgenic crops, it is considered unlikely that the Commission will override SCP's recommendation.

Avebe modified the potato to generate only the branched starch polymer mylopectin, a form of starch suited to the needs of the paper, textile and food industries. Avebe's potato is approved in the Netherlands, where the company says it will grow enough to produce up to 10,000 m.t. of mylopectin this year.

SCP found that Avebe's potato could pose a serious risk to human and animal health because a gene in the modified plant that confers resistance to the antibiotic amocacine could be transferred to humans and animals.

The SCP recommended approval for each of the 11 transgenic crops it had considered before Avebe's potato and recommended approval of a 12th: AgrEvo's herbicide-tolerant summer oil seed canola. EU approval for AgrEvo's herbicide-tolerant winter oil seed canola has been delayed by a French court. (Source: *Chemical Week*, 11 November 1998)

Plant biotechnology network launched

Reflecting the emphasis on transnational coordination and networking of FP5's quality of life and living resources thematic programme, the European Commission recently launched the European Plant Biotechnology Network (EPBN) with funding of ecu 426,000 from the biotech programme. Its aim is to promote networking between laboratories working on EU-funded research, and to facilitate the dissemination and exploitation of results. The EU currently funds 45 different projects in plant biotechnology, involving 394 laboratories in 20 countries.

The new initiative, which has been established in collaboration with the Plant Industrial Platform, the European plant science community and AMICA, a pan-European association representing plant research in the member States, has three objectives:

- The transfer of technologies from plant biotechnology research projects to industry;
- Greater interaction between researchers and end-users; and
- Promotion of the benefits of plant biotechnology.

EPBN will conduct contact meetings with industry, organize entrepreneurial workshops, operate a technology brokerage service, produce public relations material and coordinate a European Plant Biotechnology Week.

For further information contact: DG XII: S. Hogan. Fax: (32-2) 299 1860; e-mail: stephane.hogan@dg12.cec.be (Source: *Tech Monitor*, March-April 1999)

Finland

Finns focus on the future

With 50 mobile phones per 100 inhabitants and the highest number of Internet connections per capita in the world, there is no doubt that Finland is a technology-friendly nation. This is also reflected in its attitude to biotechnology. Finns are more positive about biotech than most of their European counterparts. The past decade has seen a dynamic bioscience industry spring up in and around the country's universities and science parks.

Finland, however, is a small country where human resources are limited and investment capital is scarce. Biotech consequently remains a small, albeit promising, industry.

The European Molecular Biology Organisation (EMBO) recently conducted an evaluation of molecular biology and biotechnology research in Finland. It concluded that the science was developing well and that progress compared favourably with other, larger countries.

Finland has approximately 150 biotechnology research groups based at its six universities (Helsinki, Jyväskylä, Kuopio, Tampere, Turku and Oulu). Four cities—Helsinki, Kuopio, Oulu and Turku—have science

parks dedicated to biotechnology. These "biocentres" were founded in the early 1990s and are now yielding results.

Finland as a whole has around 80 biotech firms. These have a total turnover of about ecu 400 million and employ around 3,500 people. Most of the companies are small start-ups involved in healthcare, with a few working in bioinformatics, patenting and market analysis. There are also three large, well-established industrial enzyme manufacturers.

According to management consultants Ernst & Young, Finland has the sixth-highest number of biotech companies in Europe behind the UK, Germany, France, Sweden and the Netherlands. Biomedicine and diagnostics are key areas of expertise, with a focus on preventative medicine, gene therapy, heart disease, arteriosclerosis, autoimmune diseases and cancer. A handful of start-ups work in more esoteric areas, such as the use of bovine colostrum as a raw material.

Finns are supportive of biotechnology. According to a recent public opinion survey carried out by Finnish Bioindustries, two thirds of the population believe that biotechnology will improve their lives in the next 20 years; 58 per cent think that genetic engineering is a useful technology in the development of medicines and vaccines; and 70 per cent support genetic testing to detect inherited diseases.

However, the survey also revealed that less than half of the population think that genetic engineering will improve their lives in the next 20 years, and a third believe it will have the opposite effect. More than 50 per cent think that using biotech to improve food quality is risky.

The regulatory climate in Finland is straightforward. The country has implemented two key European directives on genetically modified organisms (GMOs), and an EC proposal to amend the second directive, which covers the release of GMOs, is being discussed in parliament.

Although start-ups sometimes complain about red tape during the setting up of laboratory and production facilities or the launching of products, relations between companies and the authorities tend to be good.

Finland, like most European countries, lacks experienced biotech professionals. Although some company founders have left academia and devoted themselves to running commercial companies, many are still hanging on to their old jobs as university professors.

Finland's size and location present other problems. For a small company, selling products in the international market is difficult and expensive, and Finnish companies have to rely on collaborations with multinational companies to launch and market their products. The remote location does not help. As Genencor International's Raimo Marila points out, Finland is a fringe country and the logistics of importing raw materials and exporting products can be cumbersome.

But being small has its upside. "We have the advantages of a small country where the players in the field are limited in numbers", says Kuusi at Finnish Bioindustries. "Networking is good because people tend to know each other personally. Conflicts are easily avoided when issues can be dealt with rapidly and by direct contact."

Finns have also worked hard to build international contacts. During the post-war period, Finnish postgraduates have had excellent opportunities to visit the US and other countries and have formed close ties to many research institutions abroad. Start-ups typically have links with the US, and Finnish industry is oriented more towards the US than to the rest of Europe. Researchers are also participating successfully in joint European research projects and Japan is becoming an increasingly important scientific and business partner. (Extracted from *Chemistry & Industry*, 16 November 1998)

France

No move on maize

France's temporary ban on genetically modified maize looks set to drag on for several more months after the Conseil d'Etat (State Council) referred the matter to European Union authorities.

The Conseil, which is the country's highest administrative court, was due to decide in mid-December 1998 whether to authorize the cultivation of three varieties of genetically modified (GM) maize developed by Novartis. It now wants to consult the European Court of Justice before making the decision.

"The case raised serious legal issues at EU level which called for the input of the Court of Justice", the Conseil said. At issue is whether France can unilaterally ban crops that have been approved by the EU.

Maize producers have been awaiting a decision since September, when the Conseil slapped a moratorium on cultivation of the maize varieties after three environmental groups claimed that marketing applications filed by Novartis contained irregularities. The referral could delay a final decision for up to a year. (Source: *Chemistry & Industry*, 4 January 1999)

R-P Agro funds study

Rhône-Poulenc Agro is joining a large-scale research programme on plant genomics, called Génoplante, with the purpose of providing France with a "global, coherent and competitive" base and organization to study plant genomes and extract maximum value from the process. Two other private companies, Biogemma and Bioplante, will join the public research bodies of IRNA, CNRS, IRD and CIRAD.

The programme will benefit from a five-year, FF 1.4 billion ($233 million) budget with a little over 40 per cent of this funded by the research organizations and over 30 per cent from the three private partners. The French Government's contribution to the budget will be slightly more than 25 per cent.

The aim is to accumulate new data on the genome of large crops, identify new genes to improve plant

resistance to diseases and climate as well as identify their intrinsic features such as sugar or oil content. It is planned to screen plants that "are specific to Europe and adapted to Europe" and not be dependent on work carried out by the large multinationals.

The group's strategic committee will select the initial projects in May. However, genome analysis is likely to be carried out on maize, wheat and rape seed to acquire a competitive patent portfolio and provide seed companies with new ways of improving variety. It will also develop and coordinate work on the genomes of arabidopsis and rice. (Source: *European Chemical News*, 1-7 March 1999)

Germany

Gene bank grows seed samples

One of the largest gene banks for cultivated plants is to grow even larger. Approximately 100,000 seed samples are already stored in the cooling units of the Institute for Plant Genetics and cultivated Plant Research in Gatersleben (Saxony-Anhalt). Its takeover of the gene bank in Braunschweig now adds a further 55,0000 samples. The institute's aim is to research and preserve the genetic diversity of cultivated plants. Accordingly, staff carry out complex tests and recultivate some 8,000 plants a year. The gene bank provides approximately 15,000 seed samples a year for scientific purposes and for cultivation, for example, in botanical gardens in Germany and abroad. Information is available on the Internet at: www.ipk-gatersleben.de (Source: *Deutschland*, No. 2, 1999)

GM effects study

The German Environment Agency is to conduct a five-to-ten-year study to monitor the long-term effects of commercially planted GM crops. No GM crops have yet been fully commercialized in Germany. Oilseed rape and maize will be research priorities. (Source: *European Chemical News*, 8-14 March 1999)

India

India to join Paris Convention and Patent Cooperation Treaty

India joined the Paris Convention and the Patent Cooperation Treaty on 7 December 1998, and can now be designated in PCT applications. The Indian Patent Office is being restructured and centralized in New Delhi, and amendments to the Indian Patents Act are being proposed to permit protection of pharmaceuticals and novel organisms, including genetically-modified organisms. (Source: *Australasian Biotechnology*, vol. 8, No. 6, December 1998)

Model Indian deal generates payments

A native community and an Indian research institute will collect the first payment from a landmark agreement to market a herbal tonic derived from a local plant. The agreement is seen as a model for so-called bioprospecting efforts endorsed by the 1992 Convention on Biological Diversity.

The medicine is based on the active ingredient in a plant, *Trichopus zeylnicus*, found in the tropical forests of southwestern India and collected by the Kani tribal people. Scientists at the Tropical Botanic Garden and Research Institute (TBGRI) in Trivandrum, Kerala, isolated and tested the ingredient and incorporated it into a compound, which they christened "Jeevani"—giver of life. The tonic is being manufactured by the Aryavaidya Pharmacy Coimbtore Ltd., a major Ayurvedic drug company.

The process marks perhaps the first time that cash benefits have gone directly to the source of the knowledge of traditional medicines, says Graham Dutfield, an ecologist with the Working Group on Traditional Resource Rights at the University of Oxford, UK. "It is a replicable model because of its simplicity", he says about a chain of events that began well before the international biodiversity treaty was signed.

TBGRI scientists learned of the tonic, which is claimed to bolster the immune system and provide additional energy, while on a jungle expedition with the Kani in 1987. A few years later, they returned to collect samples of the plant, known locally as arogyapacha, and began laboratory studies of its potency. In November 1995, an agreement was struck for the institute and the tribal community to share a licence fee and 2 per cent of net profits. Another agent from the same plant is undergoing clinical tests for possible use as a stamina-building supplement for athletes.

Botanist Peter Raven, director of the Missouri Botanical Gardens, considers this agreement a "very good model for future" partnerships throughout the developing world. The current agreement must be renegotiated in 7 years, and the tribal community is expected to use the money for health care facilities and schools. (Extracted from *Science*, vol. 283, 12 March 1999)

India sequences chickpea

In an effort to boost agricultural biotechnology, India's Government has launched a plant genome initiative to sequence the entire genome of chickpea, a major Indian food crop. The Government has allocated $4 million to The National Centre for Plant Genome Research (NCPGR; New Delhi) to conduct sequencing in association with a network of seven plant molecular biology laboratories funded by the Government's Department of Biotechnology. India has chosen legumes because crops such as rice, wheat and maize are already being sequenced elsewhere and " other countries

will never take up chickpea as it is not their crop". However, attracting qualified researchers could prove tricky, as many of the country's plant genomics researchers are being lured to major industry players. (Source: *Nature Biotechnology*, vol., 17, March 1999)

Monsanto crop trials halted in India

The Government of the State of Andhra Pradesh has ordered Monsanto's local subsidiary, Mahyco Monsanto Biotech, to stop all field trials in seven districts. The local decision follows a campaign by some farmers and scientists against transgenic crops, which has involved burning Monsanto trial crops and storming its offices in Hyderabad.

Monsanto has reportedly asked the Andhra Pradesh court of Bangalore to pass a temporary restraining injunction against campaigners associated with the activist group Karnataka Rytu Sangham (KKRS) to stop demonstrations and speeches. KKRS and other activists say they are concerned by Monsanto's plans to introduce the so-called "terminator gene" in cotton to prevent farmers from harvesting seed for next year's crop.

Public resistance to transgenic crops in India is growing rapidly, mirroring activities in Europe during the past two years. (Source: *Chemical Week*, 23-30 December 1998)

Sprouting up: India's Biodiversity Act

India's Union Ministry of Environment and Forests has come up with some strongly-worded draft legislation on biodiversity aimed at preventing biopiracy of its resources. The proposed Biological Diversity Act aims to check the runaway theft of the country's genetic wealth, and also to ensure that both domestic and foreign users of this wealth do so in a manner which is sustainable and fair. The proposed Act is designed to turn the spirit of the Convention of Biological Diversity into a national instrument with real teeth. It aims to achieve three things: conservation of biodiversity, sustainable use of biological resources, and equitable sharing of benefits arising from such use. To this end, the Act:

1. Prohibits transfer of Indian genetic material outside the country, without specific approval of the Indian Government;
2. Stipulates that anyone wanting to take a patent or other intellectual property right (IPR) over material or related knowledge, must seek permission in advance;
3. Provides for the levying of fees and royalties on such transfers and IPRs;
4. Regulates access to such material by Indian nationals also, to ensure that there is some control over over-exploitation (e.g. of medicinal plants), and that there is some sharing of benefits to all concerned parties;
5. Provides for measures to conserve and sustainably use biological resources, including habitat and species protection, conservation in gene banks, environmental impact assessments of all projects which could harm biodiversity;
6. Empowers local communities to have a say in the use of their resources and knowledge, and to enter into negotiations with parties who want to use them;
7. Provides for the development of appropriate legislation or administrative steps, including registration, to protect indigenous and community knowledge;
8. Empowers governments to declare Biodiversity Heritage Sites, as areas for special measures for conservation and sustainable use of biological resources;
9. Stipulates that risks associated with biotechnology (including the use of genetically modified organisms), will be regulated or controlled through appropriate means;
10. Provides for the designation of repositories of biological resources.

The Act proposes to set up bodies at three levels (national, state and local), to carry out the above functions. Importantly, the Act provides citizens with the power to approach courts if they detect violations. Practical implementation will be a minefield if the bill makes it through parliament, but the Act presents a bold step by a national government to take the issues of biodiversity conservation and sustainable use seriously and to take steps towards addressing the thorny issues involved.

For more information, contact Ashish Kothari, Kalpavriksh, Apt. 5, Shree Dutta Krupa, 908 Deccan Gymkhana, Pune 411004, India. Tel.: and Fax: (91-212) 35 42 39; e-mail: ashish@nda.vsnl.net.in (Source: *Seedling*, March 1999)

Japan

Japanese budget boosts biotech

Biotechnology is among the beneficiaries of Japan's largest-ever budget, unveiled last December and intended to revive the nation's struggling economy through increased public spending and creation of new technology businesses.

The budget for fiscal year 1999 includes a generous 8.1 per cent increase in science and technology spending, with a particularly strong support for the life sciences. Total spending on biotechnology among five science-related ministries will total ¥284.2 billion (US$ 2.5 billion), an increase of 12.3 per cent from last year.

The budget increase is in line with the five-year plan for science and technology, under which the government has promised to double its spending on research between 1996 and 2001. Last year's budget was formed under a fiscal austerity law that called for strict budgetary restraint and spending caps to control government deficits. In contrast, the fiscal 1999 budget has seen a significant boost thanks to the government's plan to achieve a projected economic growth of 0.5 per cent in gross domestic product by the end of the fiscal year.

The Science and Technology Agency (STA; Tokyo) has benefited most from the budget increase, with a 26 per cent growth in its biotechnology-related spending, of which genome research occupies a substantial portion.

The increase reflects this year's opening of the new Genomic Sciences Center, funded primarily by the Institute of Physical and Chemical Research (GSC; Wako City), which is funded by the STA. The Center, the main site of which is due for completion in Yokohama next year, focuses on three areas of research: human and mouse cDNA sequencing and functional genomics for both humans and mice; development of a "gene encyclopedia" of the mouse genome; and the analysis of protein function and structure using nuclear magnetic resonance.

The STA has also been allotted ¥9.2 billion (US$ 81 million) for a new project encompassing bioinformatics and investigation of information processing in the brain. The project is part of a new interministerial programme bringing together life sciences and information sciences, and is supported by the one-off appropriation as part of its "social infrastructure programme". (Extracted from *Nature Biotechnology*, vol. 17, February 1999)

MITI reports on 21st C bioindustry

The Ministry of International Trade & Industry (MITI) has published its report on the 21st century bioindustry.

The report proposes that concerned government agencies cooperate in establishing a foothold for DNA analysis, intensive investment in human gene analysis, intensive investment in competitive biosectors such as metabolic engineering utilizing genes and rice genomes.

It also proposes reinforcing sectors such as pharmaceutical development and food biotechnology by harnessing high-level analytical, and information management technology from the chemical, electronics and mechanical engineering industries.

In addition, it points to the need to improve public opinion about biotechnology through supportive programmes and establishment of standards to protect genetic patents and other measures to protect intellectual property. (Source: *McGraw Hill's Biotechnology Newswatch*, 7 December 1998)

Japan biotech plan

Four Japanese Government ministries are joining forces to promote the country's biotechnology industry with the aim of creating 1,000 new companies by 2010.

Experts from the ministries of industry, agriculture, health and education have teamed up with the Science and Technology Agency to formulate policies aimed at building up the sector. A strategy is expected by the summer.

Japan currently has around 100 small biotech firms, far fewer than many Western countries. The Government now wants Japan to start competing with biotech giants such as the US and UK.

Ministers have already formulated a five-point plan. This includes support for genetic science and applied technological research, aid for biotech firms, reform of the regulatory structure and increased efforts to promote the public understanding of science. (Source: *Chemistry & Industry*, 1 March 1999)

Domestic trials abandoned

A growing number of Japanese pharmaceutical companies are carrying out clinical trials of new drugs in Europe rather than in Japan, where regulations are notoriously rigorous and testing more expensive, according to a report released in August 1998 by the Japan External Trade Organization (JETRO; Tokyo). JETRO's survey of 64 Japanese drug companies, all carrying out some kind of activity in Europe, reflects a major decline in Japan's domestic drug industry over the last couple of years as more firms seek ventures outside Japan in order to cut development costs and establish a base from which to exploit overseas markets.

Forty-seven new drugs were approved in Japan in 1994, while 24 were approved in 1996, and only 15 in 1997. Japanese firms are currently in the process of testing or awaiting approval of 70 new drugs in Europe (of which 45.5 per cent are in phase I) and a total of 56 new drugs in the US.

Factors contributing to slower clinical trials in Japan include the abolition of the drug price scheme under the National Health Insurance (NHI) system, and the introduction of the reference pricing system, which will eliminate the gap in pricing between the NHI reimbursement tariff and the wholesale price of drugs (*National Biotechnology* 16:506-507, 1998). As a result, Japan's domestic pharmaceutical market—already severely affected by the nation's economic crisis and the reform of the NHI system—is expected to face a sharp decline.

The Japanese ministry of health and welfare (MHW; Tokyo) is often reluctant to approve clinical trials for new therapies unless efficacy has already been shown elsewhere. As well as making the approval process slower than other countries, this encourages Japanese companies to launch products overseas before marketing them in Japan.

The Japanese Government's new good clinical practice (GCP) guidelines, which came into effect in April 1998 have further discouraged clinical trials. Stricter guidelines—such as requiring written instead of oral informed consent—have made it more difficult for hospitals to undertake trials, the numbers of which are falling each year. According to the ministry of education, science, sports and culture (Monbusho; Tokyo), the number of newly contracted clinical trials at 42 national university hospitals in 1997 was less than half those of the previous year.

By shifting the core of their overseas operation to Europe, many Japanese drug companies can save money via cheaper clinical trial participants, for instance, as well as having greater control over developing drugs by entering the market directly instead of licensing drug development to European manufacturers. Nine products from Japanese companies have already been approved in Europe in the past year, six of which have been developed from research to product launch solely by the Japanese company.

Although the shift overseas is likely to seriously affect the number of new drugs launched in the Japanese

market, the Japanese pharmaceutical industry says it is the only solution to compensate for the delays encountered in the domestic market. (Extracted from *Nature Biotechnology*, vol. 16, October 1998)

Republic of Korea

Human clone update

A human cloning experiment conducted by a team of researchers from Kyunghee University in Seoul, has raised so much international controversy that the Korean Government has decided to ban funding for all research involving the cloning of human embryos. The Government's decision came shortly after the researchers' announcement last December that they were the first to successfully produce an embryo from a human somatic cell. Researchers from Kyunghee University claim they cultivated an early stage embryo using an unfertilized egg and a granulosa cell obtained from a female patient who had been receiving infertility treatment at the university hospital. Although researchers say they destroyed the embryo after it reached a four-cell stage, the ethical implications of the experiment caused widespread concern among the public. However, other scientists doubt the validity of the claims, particularly as no supporting evidence has been released by the researchers, who say they are "keen to avoid further controversy". (Source: *Nature Biotechnology*, vol. 17, February 1999)

Malaysia

Malaysian company unveils new veterinary vaccines

Newcastle disease (ND) and infectious bronchitis (IB) disease are economically important viral diseases of poultry with high mortality and causes loss of appetite, respiratory and nervous symptoms, weight loss, and a drop in egg production. To help control this disease, Malaysian Vaccines and Pharmaceuticals Pty. Ltd. (MVP) has just unveiled a combined ND-IB vaccine which is approved for use in Malaysia and also registered for sale in Viet Nam and Indonesia, with the registration process ongoing in Thailand. MVP is a subsidiary of the MTDC (Malaysian Technology Development Corporation) Biotechnology holdings group and was formed in 1992 as a strategic partnership with Arthur Webster of Australia (a wholly owned subsidiary of American Cyanamid Co.), in collaboration with Universiti Putra Malaysia and the Department of Veterinary Services. In addition to the ND-IB vaccine, MVP also produces other veterinary vaccines against fowlpox, infectious bursal disease, swine fever and hog cholera, as well as providing diagnostic services for these diseases. It is hoped that MVP's products will benefit the local and regional livestock industry and also reduce the country's dependence on imported vaccines. The latter is particularly significant in view of the current economic crisis affecting the region. (Source: *Australasian Biotechnology*, vol. 8, No. 6, December 1998)

Biotechnology commercialization in Malaysia

The Malaysian Agricultural Research and Development Institute (MARDI) has established a marketing arm called MARDITECH, which is now set to boost the country's food and agriculture industry by offering 11 of its latest research findings for commercialization. The idea is for entrepreneurs, investors and venture capitalists to enter into a licensing agreement with MARDITECH before commencement of any venture under the supervision of the scientists who developed the chosen technology.

The technologies offered include biotechnology-based products, such as essential oils extraction from selected local herbs and spices, production of mannanese enzyme for converting oil palm waste into animal feed, and the bioprocessing of rice straw into poultry feed. It also includes some products and processes related to the food industry, including a packaging and handling system for prawns and a frozen complete meal process for Malaysian meals.

For further information contact: Dr. Ahmad Shafri, General Manager, MARDITECH Corp., 19-21-2 SHL Business Centre, Jalan SR 8/1, off Jalan Serdang Raya, 433000 Seri Kembangan, Selangor D.E., Malaysia. Tel.: (+60-3) 949835. (Source: *Tech Monitor*, March-April 1999)

Improved version of typhoid diagnostic test

Typhoid fever remains an important public health problem in many tropical developing countries, exacerbated in recent times by the appearance of antibiotic-resistant strains. Malaysian Biodiagnostics Research Pty. Ltd. (MBDR), Malaysia's first biodiagnostic company has recently announced the availability of a new, more rapid version of the diagnostic test kit for typhoid fever, *TyphiDot*, which they developed and marketed several years ago. Jointly developed by MBDR and Science University of Malaysia (USM), the rapid *1-Hour New Typhidot* test represents a significant improvement on the first generation test which took three hours to perform. MBDR hopes to work with the Malaysian Ministry of Health to replace the traditional Widal test with the new TyphiDot test by this year. Such a move would result in significant savings on the overall costs spent for the diagnosis of typhoid fever in Government hospitals and laboratories in Malaysia. The test is also being marketed in other Asian countries, including Bangladesh, Pakistan, Sri Lanka and Viet Nam. In terms of overall performance, MBDR has seen total sales of the TyphiDot test improve from RM 10,000 in 1995 to RM 800,000 in 1998 thus vindicating the efforts of the Malaysian Technology Development Corporation (MTDC) in promoting the commercialization of promising indigenous technologies.

Interested parties can contact Dr. Ong Kok Hai at ongkh@imc.po.my (Source: *Australasian Biotechnology*, vol. 9, no. 1, April 1999)

The Netherlands

Dutch Government to fight biotech patenting

Following a cabinet decision in October 1998, the Dutch Government, through its ministry for economic affairs, is starting legal proceedings in the European Court of Justice in Luxembourg in a bid to cancel a directive on the patenting of biotechnological discoveries.

The European Union biotechnology patents directive came into being in July 1998, after it was passed by the European parliament following its second reading in May.

The Dutch Government opposed the directive at this time and subsequently passed national legislation on biotechnology that runs counter to the directive by maintaining that plants and animals are non-patentable.

It is this conflict over national law and the requirements of European-wide implementation of the directive which is at the heart of the action. In parliament, the Greens, the right-wing protestants and parts of the socialists support the move.

The suit contains six main points of dispute, one of which concerns Article 100A of the directive on the harmonization of European law. Other objections to the directive are that there is not enough recognition of subsidiarity, the directive is unclear, and that it conflicts with international treaties on biodiversity and other issues.

In addition the directive violates the "physical integrity and self-esteem of patients", said a spokesman for the Dutch ministry of economic affairs.

However, the Dutch are expected to contest the EU legislation mainly on procedural grounds, claiming that the directive should have been passed unanimously by member States as it brought new laws into being. "We voted against the directive because we felt it went too far and should not have included patents on living beings", the spokesman added.

The biotechnology patent directive has to be implemented in national law in all member States. The Dutch Government is still obliged to implement the directive as it has "no right of postponement", despite the action.

The court action is expected to take at least two years to be resolved. (Source: *European Chemical News*, 2-8 November 1998)

Spain

Spanish dilemma

The tens of thousands of tonnes of toxic metals deposited near Doñana National Park in Spain after a dam at a mine collapsed could be removed using genetically engineered plants.

This suggestion, which has horrified environmentalists, was made at a meeting in Seville to decide how to deal with the pollution caused by the seven million tonnes of toxic mud that spread along the Guadalquivir River last year. Most of it has been mechanically removed, but heavy metals—including lead, copper and arsenic—have leached into the soil to a depth of 20 centimetres.

Victor de Lorenzo of the National Centre of Biotechnology in Madrid wants to remove the pollution with plants genetically engineered to suck more metals out of the soil. However, environmentalists headed by Greenpeace say that no one yet knows how bioengineered plants might affect the ecosystem.

Some scientists claim that natural plants and microorganisms would be just as effective. Pilar Bernal of the Centre for Agronomic Research in Murcia suggested that one hectare of the wild herb Alpine pennycress (*Thlaspi caerulescens*) could dispose of about 130 kilograms of zinc a year. The snag is that introducing foreign plants might also upset the local ecosystem. (Source: *New Scientist*, 23 January 1999)

Switzerland

No alien nation

Swiss biomedical researchers could soon face a ban on xenotransplants—the grafting of animal organs, tissues, or cells into people. On 7 February, Swiss voters approved by a wide margin a referendum giving parliament the authority to regulate xenotransplants. After the vote, Swiss president and science chief Ruth Dreifus said that government leaders will ask parliament to forbid alien transplants, except in special cases. Some scientists and biomedical companies worry that the new rules could begin a regulatory trend in Europe that would endanger proposed xenotransplant trials. Other experts, however, would welcome a ban: they fear the transplants could allow animal viruses to jump to humans, triggering new disease outbreaks. (Source: *Science*, vol. 283, 12 February 1999)

Hint at GM crop ban

Citing health and environmental concerns, the Swiss Government has turned down the first ever applications to grow experimental plots of genetically modified crops within its borders.

The Federal Environment Office (BUWAL) said there was "insufficient evidence" that the crops were safe.

The rulings, which come less than a year after Swiss citizens gave their overwhelming backing to gene technology, covered a herbicide-tolerant strain of corn and a mildew-resistant potato.

The local firm Pluess-Staufer had applied to grow the corn but was turned down over fears that its genes would spread to unmodified crops. The potato, subject of an

application by government research agency RAC, was rejected because it contained a gene for antibiotic resistance.

BUWAL stressed that it was not imposing a blanket ban on modified crops, saying other proposals would be reviewed on a case-by-case basis.

But it hinted that it would take a generally dim view of GM crops. "Swiss agriculture lives on our products' reputation for being pure and close to nature", it said in a statement. "Such gene technology experiments affect this image. This can have a far-reaching impact on our agricultural sector". (Source: *Chemistry & Industry*, 3 May 1999)

United Kingdom

UK biotechnology sector has Government boost

The UK Government is to put forward £13 million (€19 million) to back the use of biotechnology in industry. Announced by John Battle, the environment minister, the Bio-Wise initiative is intended to promote the transfer of technology from biotechnology into other sectors, and to promote this from the UK to overseas markets.

The funding will be available over the next four years. Applicants will be able to make proposals to receive grants funded by this source, and the money awarded must be further financially supported by companies themselves.

The initiative will focus on sectors where the application of biotechnology is encouraging competitiveness and sustainable development in UK industry, the Department of Trade and Industry said. The scheme is expected to be under way in six months. (Source: *Manufacturing Chemist*, March 1999)

Food retailers bow to health concerns

Three UK fast-food chains have banned transgenic ingredients. The decision comes amid growing public concern over the safety of transgenic foods. Leading UK supermarket chain Asda says it is moving toward a ban on transgenic ingredients, including transgenic derivatives, in its Asda-label foods. Several other retailers are reportedly considering similar moves.

The developments follow research at the Rowett Institute (Aberdeen, UK) finding that transgenic foods present a serious health risk. Rowett Institute head Philip James told the committee he now believes that safety checks on transgenic crops should be enhanced. Meanwhile, research by the Scottish Crop Research Institute (Dundee) shows that a transgenic potato containing the natural insecticide lectin can harm non-pest insects. The institute found that after ladybugs ate aphids that had fed on transgenic potatoes, their lifespans were halved and their reproduction was significantly reduced. (Source: *Chemical Week*, 17 March 1999)

Research on human embryo cloning

A panel of British scientists has advised that research involving the cloning of human embryo cells should be extended under very close supervision so that it can be used to treat people with brain diseases such as Alzheimer's and Parkinson's and certain cancers.

The panel also rejected the use of cloning for creating full-term human's fetuses.

A similar rejection took effect in the Republic of Korea, when scientists destroyed a human embryo that had grown to the four-cell limit, the size set by the country's scientific ethics board for human experiments.

The Korean experiment is the first reported creation of a human embryo using nuclear transfer. Had the embryo been implanted in a surrogate mother, it could have grown to full-term, producing a duplicate of the donor.

"There was very little support for reproductive cloning, though there were a few who saw benefit in certain circumstances", said Sir Colin Campbell, chairman of the Human Genetics Advisory Commission (HGAC). "The idea of human reproductive cloning is not going anywhere in this country".

But the report by HGAC and the Human Fertilization and Embryology Authority (HFEA) keeps the door open for use of cloning techniques to create tissue and organs completely compatible with a person's immune system.

The clones could be created from cells donated by the person needing the tissue or organ. The tissue to be used would then be taken from a very early term human embryo grown from the donor cell.

Cloning of human beings has been banned under British law since 1990, but the debate on clone technology was renewed by the creation of Dolly, the cloned sheep in Scotland.

Campbell ruled out any use of cloning in connection with infertility treatment. He said both scientific and public sentiment called for a clear-cut and staightforward ban on such use.

He added that, despite the ban on reproductive clones, "Cell replacement techniques might be helpful with research". (Extracted from *McGraw Hill's Biotechnology Newswatch*, 21 December 1998)

Grants available to new projects

The first details of a new competition for discretionary grants, available for innovative projects that demonstrate the use of biotechnology in industry, has been announced by UK industry minister John Battle.

The competition will form a major component of the UK Government's recently formed Bio-Wise programme and will directly help companies realize the financial, environmental and technical benefits of using biotechnology. The Bio-Wise programme will receive £13 million of DTI funding over the next four years. (Source: *European Chemical News*, 12-18 April 1999)

Panel to vet gene testing

The British Government is taking steps to prevent insurance companies from discriminating against people who have had genetic testing. Critics worry that insurers may use the tests, which can reveal who carries genes that increase disease risks, as an excuse to increase policy prices or deny coverage to those carrying "bad genes".

Government officials announced that they will work with insurers over the next year to devise a scheme for reviewing test reliability and the fair use of results in policy pricing. The initiative, led by the Government's Advisory Committee on Genetic Testing, will also establish an appeals process for those who believe insurers have discriminated against them. (Source: *Science*, vol. 282, 13 November 1998)

UK biotech centre launched

The recent launch of the International Centre for Life in Newcastle upon Tyne is expected to give a boost to the biotech sector in that region of the UK. One of the Centre's main goals is to increase the public's understanding of genetics and biotechnology.

The Centre is one of Britain's 14 linked Millennium projects and is funded by £58 million of public money (half of which is from the UK's Millennium Commission). It is unique in that it brings together five elements on one site—a state-of-the-art commercial bioscience centre, an academic department of genetics, an education facility for schools in the region, a visitors centre and a bioethics forum, all of which will be ready by April 2000. The Centre's patrons are DNA Nobel Prize winners Francis Crick and James Watson.

The Bioscience Centre offers a central cell and molecular biology facility at GMP level, a bioinformatics unit (one of the few in the UK), a biotechnology business development unit and central services, the latter two features being of special interest for new and emerging companies.

There is also the possibility of collaboration with the five-star-rated Institute of Genetics, part of the University of Newcastle that is due to move onto the site. The Institute has expertise in inherited cancers, cardiovascular and muscle disorders and prenatal diagnosis.

"We have done everything that is required to set up a state-of-the-art building for biotech, and we now want to take our place in Europe as one of the leading centres", comments Neil Sullivan, Ph.D., director of the Bioscience Centre. Dr. Sullivan is particularly keen to attract companies in the area of antiangiogenesis, antisense, apoptosis, gene therapy, tissue engineering and other key areas of therapeutics. (Extracted from *Genetic Engineering News*, 15 April 1999)

United States of America

US Congress passes "debt swap" legislation to save tropical forests

The US Government has enacted the Tropical Forest Conservation Act (PL 105-214) which permits developing countries around the world to reduce their debt to the US in return for setting up trust funds to pay for the protection of their tropical forests, of which more than 540 million acres disappeared between 1980 and 1995. The legislation, recognizing that forests are vital for all life on Earth—to purify the air, provide homes to countless species of plants and animals, protect important water systems, provide wood and medicines, and help combat global warming—addresses the issues that led to their destruction. Cutting down trees to sell for wood, one of the main forces that destroy forests, provides the hard currency developing countries need to raise their standards of living and pay off debts owed to industrialized countries. In 1998, many forests in Indonesia, Brazil and Mexico, were destroyed by forest fires, contributing to the 40 million acres destroyed each year.

The bill, which obtained strong bi-partisan support and was signed by President Clinton on 29 July 1998, was based, in part, on the World Wildlife Fund's experience of brokering debt-for-nature swaps that have acquired more than $55 million of commercial debt from nine countries since 1987. The legislation was protected from amendments that would have diluted its purpose and possibly prevented its passage.

The Tropical Forest Conservation Act of 1998 establishes a Tropical Forest Facility in the Department of the Treasury to administer the Act. The Facility benefits eligible developing countries with tropical forests, primarily in Latin America and the Caribbean, by reducing or cancelling loans or credits, in support of activities to preserve or restore tropical forests. The funds provided by the Act are to be used to provide grants to non-governmental, environmental, forestry, conservation, and indigenous peoples organizations in the beneficiary countries (Source: *Diversity*, vol. 14, Nos. 3 & 4, 1998)

Biotech seeds strong growth

Demand for agricultural biotechnology products in the US is projected to increase 27 per cent/year to $2.9 billion in 2002, according to a new study, "Biotechnology in agriculture", from Freedonia.

Major growth will be in the transgenic crop segment as demand for insect-protected and herbicide-tolerant crops increases. The imminent commercialization of new genetically enhanced crops will add further value to the agro/biotech market.

Transgenic seeds and plants will remain the largest and fastest growing segment by value, accounting for 70 per cent of total demand in 2002. Cereal grains and oil crops will constitute the overwhelming majority of demand.

Freedonia points out that several factors may exert a moderating influence on the market. For example, if pests resistant to the modifications inserted into transgenic crops emerge, farmers may be forced to revert to conventional crop protection methods. The possibility of a consumer backlash against certain products remains real, especially with continued scepticism towards genetic engineering in Europe. (Source: *European Chemical News*, 22-28 February 1999)

D. RESEARCH

Research on human genes

Stem cell studies

Once considered an impossible dream, embryo stem cell research is becoming biotechnology's hot new field as laboratories race to create therapies out of the cells that appear fleetingly at the earliest stages of life.

They hope to learn how to grow these primordial cells in the laboratory, and then coax them into becoming all kinds of tissues, from bone marrow to neurons.

Ultimately, scientists think this work will lead to human patch kits that can replace virtually any worn out body part.

Two groups this month published studies on cultivating these cells. A third group later announced that it used cloning to create human embryonic stem cells from the fusion of an adult cell and a cow egg.

As many hailed the research, some experts pointed out that the breakthroughs reported in the past month are only the beginning. There are many technical barriers to cross before these cells will make it to the clinic.

Others fear these methods present serious ethical issues that must be sorted out.

"There are a lot of different techniques moving forward, from several different companies, because the potential is so enormous", said Princeton University molecular geneticist Lee M. Silver.

"The field is exploding", said Silver.

Silver, who is the author of "Remaking Eden: Cloning and Beyond in a Brave New World", said the group that creates a way to produce embryonic stem cells in the laboratory will have a tool to manufacture everything from bone marrow to nerve cells to hearts. It would be a goldmine.

He predicts the simplest therapy, regrowing bone marrow for transplants, could come in as little as five years.

"I have no question that one of these techniques is going to work", he said.

One company that has already benefited from the quest is Geron Corp. The Menlo Park, CA Company was the financial muscle behind two laboratories—at the University of Wisconsin-Madison and at Johns Hopkins University in Baltimore—that reported the isolation and cultivation of human embryonic stem cells in the first week of November 1998.

Thomas Okarma, vice president of R&D for Geron, said that it may be possible to genetically engineer these cells to elude attack by the immune system, overcoming one of the major problems faced in bone marrow transplants and other kinds of organ and tissue donation. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 16 November 1998)

Does Alzheimer's bully brain cells?

The mental decline of Alzheimer's disease may have a physical rather than a chemical cause, say researchers at the University of Tasmania.

Most researchers are pursuing the idea that the plaques of amyloid protein that form in the brains of Alzheimer's patients are toxic. This poisoning supposedly leads to the formation of abnormal tangles of other proteins in the cytoskeleton, which gives nerve cells their shape. These tangles are thought eventually to kill the cells.

But James Vickers and his colleagues suspected that the physical presence of the plaques might be just as important. They used a needle to damage a small part of rats' brains and discovered that the changes this triggered bore a marked resemblance to those in the brains of 20 patients in the earliest stages of Alzheimer's disease. Viewed under an electron microscope, it was found that a cytoskeletal protein called neurofilament had lost its normal string-like structure and instead formed clumps and rings.

The rats' damaged neurons also started to sprout new extensions, in much the same fashion as the cells surrounding plaques in the brains of Alzheimer's patients. However, the rats' brains did not have plaques.

Vickers speculates that plaques of amyloid protein set in train a cycle of futile attempts at nerve cell regeneration, causing tangles of neurofilament protein to form. If Vickers's theory is correct, it might be possible to treat Alzheimer's using drugs that stabilize cytoskeleton proteins, such as the anti-cancer drug taxol. (Source: *New Scientist*, 5 December 1998)

Naked DNA increases IFN levels

Researchers from Vical Inc. have reported the first effective delivery of a cytokine gene to stimulate the systemic production of a powerful anti-cancer protein, in an experiment involving mice injected with tumours in a process known as "naked DNA transfer".

In a series of two experiments, researchers were able to show decreased rates of tumour growth, and increased rates of survival through the injection of these immune system-stimulating genes.

Naked DNA transfer involves the direct transfer of synthetic DNA formulations into cells without a vector. The scientists believe this can bypass some of the problems that have plagued the field of gene therapy.

Previously, researchers have introduced a synthetic gene into the nucleus of cells through the viral vectors, genetically crippled viruses equipped with the gene within a lipid cell or liposome, but both methods have met with serious drawbacks.

In the first experiment, melanoma tumours were implanted beneath the skin of mice. The mice were then treated by the injection of the DNA into a leg muscle.

In 39 days, 90 per cent of treated mice were still alive, while only 10 per cent of the control group survived.

Another experiment tested the treatment approach against metastatic melanoma.

Tumour cells were injected into the tail vein of mice and after 25 days, 80 per cent of the treated group were found to have no significant spread of tumour growth.

Some 70 per cent of the control group, those not treated with the naked DNA transfer, were found to have tumorous nodule growth far too numerous to count.

Circulating levels of interferon were measured during both pre-treatment and post-treatment stages using standard laboratory procedures.

The control animals were also measured and, along with the pre-treatment stage mice, were found to have undetectable levels of interferon alpha.

It took several injections of the synthetic gene to produce measurable levels of interferon.

Mr. Allan Engbring, director of investor relations for Vical, said this new technique represents a significant improvement over delivering interferon to cells intravenously.

"In order to have an effective dose, you (had) to deliver a lot of it into the bloodstream", Engbring said. "So you are putting a lot of it into the bloodstream to get to where you need it, to do the job against the cancer. The limiting factor on interferon is how much you can tolerate".

Researchers have attempted to use interferon to treat diseases for decades. Though naturally produced by cells in response to viruses and tumours, attempts to externally manufacture interferon and reintroduce it into the body have met with limited success.

Harmful side effects, such as flu-like symptoms and anaemia, coupled with the high cost of obtaining sufficient quantities, have led to some disappointment over a drug that once held great promise. Some researchers think that interferon's best days lie ahead. (Source: *McGraw Hill's Biotechnology Newswatch*, 7 December 1998)

Despite the latest breakthroughs, lab-grown organs are still a long way off

It has been hailed as a medical revolution. Two teams have developed methods of culturing human embryonic stem cells, which they hope will lead to ways of growing tissues or even organs for transplants. But much needs to be done to prove that their cultures have any therapeutic value—and that tissue grown in this way would be safe.

Embryonic stem cells can differentiate to form any of the body's tissues, such as skin or muscle. But keeping human embryonic stem cells dividing in culture proved difficult. Now that obstacle seems to have been overcome.

John Gearhart of Johns Hopkins University in Baltimore isolated his stem cells from aborted foetuses. James Thomson at the University of Wisconsin, Madison, obtained his from embryos created by IVF and grown for about five days until each developed into a hollow ball of cells called a blastocyst.

Thomson and Gearhart eventually succeeded by growing the stem cells over a "feeder" layer of irradiated mouse cells. "Take the feeder cells away, and the stem cells start to differentiate", says Gearhart. "But we don't know why".

In mice, embryonic stem cells are used for genetic engineering. After their genes are altered, they are injected into an embryo which develops into a chimera that has both engineered and unaltered cells. Pure engineered mice can be bred from these chimeras.

Thomson and Gearhart do not want to create transgenic people. Their goal is to grow cells and tissues to reinvigorate diseased or ageing bodies, perhaps by combining their methods with cloning. For example, cells could be taken from a person, used to clone a blastocyst and thus obtain stem cells. Tissues grown from stem cells taken from the blastocyst would match that person's own tissues—eliminating problems with rejection.

Turning such dreams into reality will not be easy, however. No one knows how to make a stem cell differentiate to form a specific tissue.

Gearhart and Thomson cannot even be sure that their cells really can form any tissue. Ethical concerns mean that they cannot perform the acid test: deliberately creating a human chimera. The cells do have many of the right properties, however. They possess high levels of telomerase, an enzyme needed for cells to divide indefinitely, and they can form cells representative of the three main types of tissue: endoderm, mesoderm and ectoderm. But some differentiated cells, such as those from mouse yolk sacs, also pass this test. (Source: *New Scientist*, 14 November 1998)

Will cellular creations spin out of control?

Being able to keep embryonic stem cells dividing in culture indefinitely may allow biologists to grow tissues for transplantation. But can we be sure that these cells will stop dividing once transplanted? The latest research

suggests that such tissues might have changed in ways that make them more likely to become cancerous.

Most of our cells divide only a few times before they die. Researchers believe that stem cells avoid this fate because they possess an enzyme called telomerase. This repairs the tips of chromosomes, which otherwise shorten each time a cell divides. Earlier this year, Californian researchers showed that normal cells can keep dividing if given active copies of the telomerase gene.

But researchers in Seattle report that for telomerase to keep human skin cells dividing indefinitely, an anti-tumour protein called p16 must also be inactivated.

Clearly, human stem cell lines grown in culture for long periods will have to be screened for dangerous changes before being used as tissue grafts. This will be a huge task, given the number of genes that must be screened. Tom Ikarma of Geron, a company in Menlo Park, CA, which is licensed to work with human stem cells, says that no problems have emerged with mouse stem cells. But he adds that rigorous safety testing "will be part and parcel of our development of this technology". (Source: *New Scientist*, 14 November 1998)

Happy gene

A defence against manic depression may be written in our genes, scientists say.

Edward Ginns and his colleagues at the National Institute of Mental Health near Washington D.C. looked at the genes of people from families with a genetic predisposition to manic depression. Those who did not develop the disease, despite their high risk, had similarities in one region on chromosome 4. This may contain a gene that protects against the disorder.

This is the first evidence of a beneficial gene linked to a behavioural disorder. Understanding how the gene offers protection could lead to new drugs for depression that mimic its effect. (Source: *New Scientist,* 9 January 1999)

Signal failure

The inability to make just one of many immune signalling molecules called cytokines can render a person vulnerable to infection by bacteria that are usually harmless.

An international team of researchers studied a child with an inherited gene defect that prevents production of a cytokine called interleukin-12. The child has suffered serious infections from types of mycobacteria that normally only cause illness in people who have severely compromised immune systems.

The results support earlier evidence that IL-12 is crucial in warding off mycobacteria, a group that includes the organism that causes tuberculosis. Team member Jean Laurent Casanova of the Necker Children's Hospital in Paris thinks some people might be more susceptible to TB than others as a result of such gene defects. (Source: *New Scientist*, 9 January 1999)

British find gene that, like p53, may help trigger half of all cancers

A gene that, like p53, appears to play a pivotal role in triggering more than 50 per cent of all cancers has been pinpointed by British scientists.

The team from London's Institute of Cancer Research announced the discovery of Bcl10 just days after the New Year. The discovery may prove to be a major target for the development of cancer-fighting drugs.

"This is only the second gene to be discovered which is implicated in such a large number of cancers", said Martin Dyer, leader of the scientists who made the discovery.

Dyer was investigating the gene because its mutated form was thought to be the trigger for B-cell non-Hodgkin's lymphoma. But Dyer and his co-workers found that the mutation was present in many other cancers, including the most common types—lung, breast and colon.

Not since the discovery of the p53 gene, also mutated in about half of all malignancies, has such a broad cancer gene been spotlighted.

"Our preliminary results indicate that Bcl10 is contributing to the development of at least as many [different cancers] as p53", Dyer said.

"Before the isolation of p53 very little was known about the cancer mechanism. It looks as though the discovery of Bcl10 will be equally important to our understanding of the disease", Dyer added.

Dyer said he believes the discovery will play a major part in finding effective treatment for "big" cancers, such as lung tumours, that kill the bulk of the victims of the disease.

Dyer found the gene while analysing a tumour from a man who had entered the hospital with chest pains. The patient was later found to have B-cell lymphoma of the stomach. Closer examination found that the patient had a rate translocation between chromosome 14 and chromosome 1. That meant part of chromosome 14 had attached itself to a part of chromosome 1.

Dyer said that it quickly became clear that the mutated Bcl10 on the fused chromosome 1 was preventing natural cell death—apoptosis.

"At first we thought that the mutated Bcl10 gene behaved in the same way as mutated Bc12", Dyer said, referring to a gene that prevents cell death in another type of B-cell lymphoma.

But further investigation found that Bcl10 acts very much the way p53 does, preventing cell death while speeding the growth of cancer cells.

After that, Dyer's team began to look for Bcl10 in other tumours, starting with mesothelioma, a malignancy that shows abnormalities in chromosome 1, and found mutated Bcl10 genes in the tumours.

"This is very exciting and shows the value of studying rare cases", said Dyer. He had no estimation of

how long it would be before the discovery would translate into a testable therapy. (Source: *McGraw Hill's Biotechnology Newswatch*, 18 January 1999)

Adults may have all the cells needed to regenerate their own tissues

Using a patient's own tissue to grow replacement organs could be easier than anyone imagined, judging by the ease with which scientists have turned adult brain cells into blood.

Such a dramatic identity switch was thought to require nuclear transfer, the technique that made the cloning of Dolly the sheep possible, which involved stripping an egg cell of its own genetic material and replacing it with the transplanted nucleus of an adult cell.

Now an international team says that simply injecting the brain's neural stem cells (NSCs) into the bone marrow of mice is enough to promote this metamorphosis. If the same is true in humans, the technique could lead to new sources of perfectly matched transplanted tissue—without the controversial use of human embryonic stem cells, which are taken from aborted foetuses or discarded IVF embryos.

Until two years ago, the process of specialization, in which ES cells change to form individual tissues, was considered irreversible. Then Dolly's creators at the Roslin Institute near Edinburgh showed that the developmental potential of an adult cell could be recovered. They think factors in the egg "reprogramme" the cell's genes to an embryonic state so that it can form any kind of tissue. It was from a reprogrammed udder cell nucleus that Dolly was born.

Angelo Vescovi of the National Neurological Institute in Milan, Italy, says his team suspected that some reprogramming might happen without cellular surgery. They thought that NSCs, the least specialized cells in the brain and the basis of all the brain's different cell types, were likely candidates. Vescovi's team injected NSCs from adult mice into the bone marrow of mice that had been irradiated to cripple the cells that form blood, hoping that this new environment might trigger reprogramming.

Sure enough, after five months, the recipients developed new blood cells. Genetic analysis confirmed that many of these cells were direct descendants of the NSCs. Intriguingly, the NSC recipients took a month longer to recover their blood cells than a control group of irradiated mice that had received bone marrow.

This time lag fits nicely with the idea that cells are reprogrammed, says Vescovi. It suggests that the cells must first reverse their development to a near embryonic state before they can develop into the new tissue. His latest results suggest the new blood cells are functional: the irradiated mice that received the NSC transplant lived longer than the irradiated mice that received no transplanted cells.

Vescovi believes it may be possible to use stem cells from other tissues such as skin as the source of new tissue. These would be easier to obtain that the brain stem cells used in his work so far. "This way each patient, rather than an embryo, would be the source of the cells that heal them", he says. (Source: *New Scientist*, 30 January 1999)

Fool an egg into thinking it's fertilized, and it will repel all comers

A new generation of contraceptives could be on the way now that researchers have identified a receptor on the surface of eggs that binds to a sperm surface protein. By targeting such receptors, it might be possible to trick an egg into believing it has been fertilized, making it change its outer coat to keep sperm out.

Existing birth control methods, such as barrier contraceptives and hormones, all have their drawbacks. So researchers have turned their attention to biochemical interactions between sperm and eggs. Blocking these interactions could prevent fertilization. So far, scientists have identified at least three binding proteins on the sperm.

But identifying the corresponding receptors on the egg has been difficult, partly because eggs are harder to come by. Now, for the first time, Nicole Sampson and Hui Chen at the State University of New York in Stony Brook, have found a receptor for a critical sperm surface protein called fertilin-beta. In mice with a faulty fertilin-beta gene, sperm rarely fuse with eggs. The team synthesized the part of the sperm protein thought to bind to the egg, attached a radioactive tag, and then mixed the tagged peptide with mouse eggs.

The tagged fragment bound only to a receptor called alpha-6/beta-1 integrin.

Some evidence from frog eggs hints that an integrin receptor can activate the egg and prompt it to change its outer coat to prevent more than one sperm from getting in.

If this happens in humans, the integrin receptor could be a target for a new contraceptive that deceives the egg.

But so far, researchers have failed to activate mammalian eggs by targeting integrins. (Source: *New Scientist*, 23 January 1999)

Is this the mother of all brain cells?

Cells in the brain that neurologists thought were mere structural supports could turn out to be the key to future treatments for degenerative brain diseases. Scientists in Sweden have shown that ependymal cells do more than simply separate the fluid that surrounds the brain and spinal cord from neural tissue. They may, in fact, contain the brain's reserve of stem cells.

Stem cells go on to develop into mature cells, which in the brain include neurons and various types of supporting cells called glia. It was long believed that only embryonic brains had stem cells, which would mean that unlike bones or blood, adult brains could not regenerate. But in the past few years, scientists have shown that adult brains can also sprout new neurons, suggesting that neural stem cells do exist, though no one knew which cells they were.

For Jonas Frisén and his colleagues at the Karolinska Institute in Stockholm, ependymal cells were the prime

suspects. Earlier studies had shown that a gene called *nestin* is expressed in these cells following spinal cord injuries, in regions of the brain where cells are regenerating, and in the developing embryo. "It's hardly ever expressed in the adult system", says Frisén.

To test their hunch, the researchers took a number of ependymal cells from rats' brains and cultured each one separately. More than 6 per cent of the cultures developed all other major types of brain cell, the hallmark of stem cells.

Why many of the cells did not mature in this way is unclear. Frisén says it could mean that not all ependymal cells are stem cells. Or perhaps they are all stem cells but only at certain times. Frisén adds that other stem cells might also exist. Scientists hope that some day it might be possible to use a patient's own stem cells to repair damage caused by diseases such as Parkinson's, Altzheimer's or strokes. (Source: *New Scientist*, 16 January 1999)

IL-2 boosts cancer vaccine

Researchers developing a cancer vaccine based on dendritic cells have substantially improved the vaccine's efficacy using the cytokine interleukin-2 (IL-2), suggesting that future cancer therapies might rely on exploiting the natural regulatory pathways of the immune system. The team had previously developed a technique in which host dendritic cells are exposed to tumour cell lysates, then reintroduced into the host. Because dendritic cells present the antigens to T-cells to initiate an immune response against the tumour, the scientists reasoned that IL-2—a T-cell growth promoter that has already been approved for clinical use—might help the vaccine produce a more robust response. Tests of the new approach in mice showed that the combined vaccine can render the animals immune to lethal tumour challenge and cause the regression of micrometastases in mice with established tumours. According to James Mulé, a professor in the Department of Surgery at the University of Michigan and the senior author on the new study, a phase I trial on the dendritic cell vaccine has shown that "small numbers of dendritic cells can prime patients to react strongly to that antigen when presented by dendritic cells", and that the cells have low toxicity. Phase II trials on the combined vaccine are to begin in the next few months. (Source: *Nature Biotechnology*, vol. 17, April 1999)

Cellular chemo pump undermines treatment for breast cancer

A newly discovered protein that pumps anti-cancer drugs out of tumours may lead to new treatments for some forms of the disease, say scientists at the University of Maryland Greenebaum Cancer Center.

Led by Drs. Austin Doyle and Douglas Ross, the Maryland researchers reported their findings in the 22 December issue of the *Proceedings of the National Academy of Sciences*.

Ross said the findings may lead to better ways to target chemotherapy to individual patients and could open the door to new ways to thwart proteins that resist cancer-fighting medications.

Ross and his colleagues call the newly-discovered pump the Breast Cancer Resistance Protein (BCRP). They discovered that this protein pumps three common anti-cancer drugs out of cancerous cells very rapidly—before drugs can reach the nucleus of the cancer cells and destroy them.

The scientists studied resistant and non-resistant cancer cells to find genes that were different among the two groups.

"We have found that some cases of leukaemia seem to express a considerable amount of this gene as well, so our future research is going to involve detecting what cancers use this mechanism to become resistant to drugs", Ross said.

He said the gene was found in drug-resistant human breast cancer cells, but only in test tubes.

It has not yet been shown that the gene causes resistance in actual human breast cancer, Ross said, but there is every reason to believe it does, since it does in test tubes.

The hope is that "we will now be able to make antibodies so that we will be able to identify the expression of this gene".

A compound has been produced by Wyeth-Ayerst Laboratories Inc. that inhibits the pumping-out action of the Breast Cancer Resistance Protein, Ross said, but clinical trials using the chemical have not yet begun.

Ross said more animal toxicology studies are needed before Wyeth-Ayerst can continue to develop its protective compound.

"This finding helps us understand how cancer cells resist the effects of chemotherapy", Ross said.

He said that "typically" most cancers respond, at least for a short period, to chemotherapy, and breast cancer is a prime example.

But usually, he said, the cancer is not cured, but arrested for a period of time, only to return later, even under the same drug regimen that slowed it down at first.

"This is another step forward in our understanding of how cancer cells resist the effects of chemotherapy, particularly how they learn to be resistant", Ross said.

As more of these "protective mechanisms" are known, he said, "we may be able to find ways to circumvent them" and make chemotherapy more effective. (Source: *McGraw Hill's Biotechnology Newswatch*, 4 January 1999)

Human form of Methuselah yeast gene raises hope of longevity drug

Scientists say they have found a human version of a gene that increases lifespan by about 60 per cent in yeast, a finding that may lead to longevity drugs.

The researchers found the gene first in yeast, and then after years of searching, pinpointed a similar gene, which they call LAG1, in humans.

Molecular geneticist S. Michal Jazwinski said he is now conducting DNA tests, looking for LAG1 in blood samples from people who have passed their 100 birthdays.

He plans to compare the LAG1 gene of these extremely old people to the form of the gene in younger people to see if there is something different about DNA of the aged that lets them live longer.

The scientists found that by manipulating LAG1, they could extend the lifespan of the microbe by about 60 per cent. In humans, that could mean stretching life expectancy from 75 to 120.

Jazwinski has been looking for the human homolog ever since he found the yeast gene. A break came when the scientists found the longevity gene in the roundworm *Caenorhabditis elegans* a few years later. Once they had the worm version of LAG1, they were able to track it down in humans, said Jazwinski.

The investigators tested the gene's ability to control longevity by taking the DNA out of a human cell and inserting it into mutant yeast that had its own LAG1 removed, along with another gene that works along with LAG1 to regulate lifespan called LAC1. Yeast missing both these genes cannot survive.

When the scientists inserted the human LAG1 and LAC1 genes, the yeast lived about 85 per cent of a normal lifespan, said Jazwinski.

Yeast carrying the human genes have shorter lives because the DNA is not designed to work in microbes, he said.

His team is now tinkering with the human gene to see if it can be modified to allow yeast to have a normal, or even longer-than-normal, life.

Jazwinski works with yeast because it is a good easy laboratory model system on which to conduct longevity experiments. Also it would be impossible to conduct similar studies on humans.

He has, however, started studies investigating LAG1 in genetically altered mice.

Jazwinski says his finding is early-stage research, and scientists do not know why or how the protein produced by LAG1 affects longevity, but he hopes his work will one day lead to a pill that can add not just years, but healthy years to a person's life. (Source: *McGraw Hill's Biotechnology Newswatch*, 4 January 1999)

Blood vessel growth factor genes seen as aid for failing hearts

Gene therapies for coronary artery disease using two growth factors that promote angiogenesis have been shown in new studies to be safe and possibly effective.

One study, published in the journal *Circulation*, was led by Jeffrey Isner of St. Elizabeth's Medical Center in Cambridge, MA.

The study demonstrated that naked DNA encoding vascular endothelial growth factor (VEGF), injected into five men for whom conventional therapy for angina and ischemia had failed, reduced symptoms for all of them.

There was also objective evidence of improved myocardial perfusion as demonstrated by SPECT scan and reduced use of nitroglycerin.

Dr. Isner called the Phase I study "the first evidence of a favourable clinical effect of direct myocardial injection of naked plasmid DNA encoding for VEGF".

The growth factor was injected via a minimally invasive chest wall incision.

"Patients in this study experience a decrease in anginal frequency and severity, with all patients experiencing relief beginning 10 days post-gene transfer", said Isner.

All patients in the study entered with functional class 3 or 4 exertional angina that was unchanged by maximal medical therapy, areas of viable but under-perfused mycoardium, and coronary artery disease in multiple blood vessels.

Two patients' angina completely disappeared, and a third who had had daily experiences of pain had only two episodes between day 30 and 60 of follow-up visits.

The fourth and fifth patients continued to experience occasional angina but with reduced frequency and at much higher levels of activity, said Isner.

Use of nitroglycerin for all decreased from over seven tablets to about one per day by day 60 after the genes were infused.

Remaining tissues that need to be resolved concerning gene therapy for CAD include determining the best site for injection, and the number and dose of the injections, said Isner.

Which vector is best, and indeed which growth factor is the best, remains to be determined in future trials, he added.

Earlier this year, German researchers led by the University of Freiburg demonstrated that recombinant FGF-1 was able to induce the growth of blood vessels in the heart in 20 patients with three-vessel coronary disease.

Angiography showed that new vessels and capillaries grew in all cases around the injection site which bypasses blocked arteries. The trials used FGF-1 in conjunction with surgical angioplasty. The researchers say the two treatments could be used together in patients for whom surgical opening of blocked vessels could not be done successfully. (Source: *McGraw Hill's Biotechnology Newswatch*, 4 January 1999)

The body's anticancer weaponry backfires in old age

A protein that helps the body fend off cancer appears to cause the immune system to degenerate in old age. Researchers in California say that as the body ages, protective cells of the immune system become hyper-sensitive to the protein, and die at higher rates.

Many cells in the body produce tumour necrosis factor (TNF), a protein that helps to destroy diseased cells by triggering the cell's self-destruct mechanism. Most cells ignore the suicide instructions, and even switch on protective mechanisms when exposed to TNF. But infected or cancerous cells should obey the signal, setting

loose proteins called caspases that dissolve the nucleus of the cell.

As people age, levels of TNF in their blood rise. Immunologist Sudhir Gupta of the University of California at Irvine wondered if the protein might account, at least in part, for the weakening of the immune system that also comes with age. He and his colleagues collected blood samples from 15 retired professors between the ages of 65 and 95 and from 15 students and staff between 20 and 29. From the samples, they cultured two types of T cells, CD4 and CD8, which work on the immune system's front line to destroy invading or infected cells.

When exposed to TNF, 26 per cent of the CD8 cells from young subjects underwent apoptosis. By contrast, 40 per cent of the aged CD8s committed suicide. The researchers found a similar increase in cell death among the older group's CD4 cells, suggesting they had become more sensitive to TNF.

Gupta then counted how many T cells from each group had a surface receptor for TNF. These come in two types. Receptor I reads the apoptosis signal from TNF, while receptor II ignores it. Gupta found that in the young volunteers, far more T cells had type II receptors than type I. But the opposite was true in the older subjects.

Gupta says that TNF and T cells are not the whole story. He says rates of apoptosis probably increase in many other cell types as well, and many other signals besides TNF are involved. His lab is now studying other cell-death mechanisms.

Gupta's ultimate goal is to discover ways to alleviate some effects of old age. He says drugs that target caspases may help preserve the ageing immune system. (Source: *New Scientist*, 20 February 1999)

Panning for Taxol-binding proteins

New work by scientists from Florida State University, Tallahassee and the University of London's Birkbeck College provides further insight into the mechanism of action of the anticancer drug Taxol and could facilitate the design of new drugs. The cytoskeletal protein tubulin has long been known as a cellular target for Taxol, but it has been unclear how this interaction results in the death of cancer cells. Lee Makowski and his colleagues set out to identify other cellular targets of Taxol to gain better insight into how it works. The team screened a 12 amino acid phage-displayed peptide library of cellular proteins and identified over 70 peptide clones with affinity to Taxol. After sequencing the clones and searching for similarities to human protein sequences, they found that one-third had significant similarity to the disordered loop region of the BCL-2 protein, which is involved in apoptosis. ELISA-binding assays showed that the selected peptides were predictive of Taxol-binding sites in BCL-2. The group is characterizing additional clones identified in the screen and plans to investigate further how the drug's interaction with BCL-2 is involved in killing cancer cells. (Source: *Nature Biotechnology*, vol. 17, February 1999)

Breathing easier with AAT deficiency

Deficiency of α-1-antitrypsin (AAT) protein is responsible for approximately 3 per cent of all early deaths due to pulmonary disease. Gene therapy has been suggested as a possible treatment, but it has not been clear whether AAT can be delivered at therapeutic levels. Terence Flotte and colleagues at the University of Florida, Gainesville have reported the development of a recombinant adeno-associated virus (rAAV) that is capable of expressing human AAT at therapeutic levels in mice. Injection of the rAAV-hAAT virus into the muscles of two different strains of mice resulted in greater than 800 μg hAAT per millilitre of serum. The human AAT protein was expressed at consistently high levels for three months. High serum levels of AAT are crucial to the success of any gene therapy approach for AAT deficiency, as protein replacement therapy requires weekly intravenous infusions to maintain high enough serum levels to combat the onset of pulmonary disease. Scaling up expression to account for the size difference between mice and humans is the next step, says Flotte. (Source: *Nature Biotechnology*, vol. 17, February 1999)

Acetaldehyde suspected as trigger for cancer

The same chemical that causes a hangover after a hard night's drinking may also cause cancer, according to a new report by scientists at the State University of New York at Stony Brook, Fox Chase Cancer Center, Philadelphia, and Japan's Kyoto University. Acetaldehyde is produced by the body as it metabolizes alcohol—specifically, ethanol—and is the culprit in that "morning-after" feeling, said Shinya Shibutani, Ph.D., in a study in the journal *Biochemistry*, published by the American Chemical Society.

While the body normally metabolizes small quantities of alcohol via the enzyme aldehyde hydrogenase 2, some people—most notably women and most Asians—do not produce enough of this enzyme to process ethanol.

Asians also seem to be particularly vulnerable to cancers of the esophagus and liver. The new research suggests that "prolonged alcohol intake beyond the capacity of detoxification of acetaldehyde in the body may increase cancer risk".

Specifically, the study shows that an aldehyde-damaged nucleotide, N2-ethyl-deoxyguanosine, or N2-ethyl-dG, is readily incorporated into mammalian DNA.

This lends support to the observation that heavy drinkers and Asians may suffer from higher-than-average occurrences of cancers of the esophagus, larynx and liver.

Acetaldehyde is also produced during the normal digestive process, and is found in many foods as well as in car exhaust and cigarette smoke. It is also produced during industrial processes used in making plastics, dyes and synthetic rubber. (Source: *McGraw Hill's Biotechnology Newswatch*, 1 February 1999)

Italian bio-alchemists turn brain stem cells into blood

Stem cells taken from adult brains have been turned into blood cells in a scientific breakthrough that could avoid ethical quandaries posed by the use of human embryos as the source of building blocks for cell therapy.

The finding opens the door to research that could lead to organ repair kits made with cells taken from an adult's own body, said neurobiologist Angelo Vescovi, who led the research team from Milan's National Neurological Institute Carlo Besta in Italy.

If it works, the new method could bypass the controversial use of human embryos as the source of stem cell, the versatile base material for all human tissues, he said.

His experiment, published in the journal *Science*, shows for the first time that some stem cells can retain their "plasticity"—ability to take on new identities—even after a body matures, said Vescovi.

Vescovi worked with stem cells from the brains of adult transgenic mice. He used neural stem cells, which scientists have thought could grow into several kinds of brain cells, but not other body tissues.

After isolating the cells, he grew them in culture dishes and then injected them into the tail veins of mice whose bone marrow had been nearly destroyed by radiation.

When the scientists examined the mice five months later, they found new blood and bone marrow, composed of cells bearing the genetic fingerprints of the donor animals.

"We have brain cells making blood", Vescovi said.

In an accompanying article in the journal, neurobiologist Ron McKay of the National Institute of Neurological Disorders and Strokes, Bethesda, MD said that the research opens the door to using these brain cells to replenish bone marrow for the treatment of blood diseases. He added that neural stem cells grow more readily than bone marrow stem cells in the laboratory.

Studies are under way in his own laboratory and in others around the world looking at stem cells from grown animals. His team has turned brain cells to muscle, for example, and another has worked with bone marrow cells, turning them into brain cells.

These studies are suggesting, he said, that "animal cells and animals are much more dynamic than people had thought".

More research is needed to show if what works for mouse brain cells will also work for humans, and what molecular cues cause the switch, said Vescovi. So far, the Italian team does not know why these brain cells turned to blood.

Also, he said other experiments will be needed to show how effectively different cells—like skin cells—can be coaxed to take on a new identity. (Source: *McGraw Hill's Biotechnology Newswatch*, 1 February 1999)

Mitochondrial "Eve" older than thought

Mitochondrial Eve, from whom all women are descended is twice as old as we thought, according to the suggestion from two new studies that challenge the idea that mitochondrial DNA (mtDNA) is inherited only from our mothers.

Mitochondria, the energy-generating structures in all our cells, have their own DNA. Even if mitochondria from sperm get into the egg during fertilization, biologists thought they would be rapidly eliminated.

This simple pattern of inheritance, as well as the speedy rate at which some parts of mtDNA mutate, has made it a very useful molecular clock to study evolution. Using this clock, biologists were able to estimate that "Eve", the most recent common ancestor of all women, lived in Africa between 100,000 and 200,000 years ago. Tracing "Adam", the equivalent common ancestor for men, has proved more difficult.

But two studies of the distribution of mtDNA mutations in human populations now suggest that there is a degree of paternal mtDNA inheritance. That throws previous evolutionary calculations using mtDNA into confusion and greatly complicates the business of estimating when Eve lived.

In the first of these studies, an international team led by Erika Hagelberg of Cambridge University studied the distribution of one mtDNA mutation on the island of Nguna in the Melanesian archipelago of Vanuatu. The mutation, which causes the substitution of a single amino acid in one mitochondrial protein, is present in people of many separate maternal mtDNA lines.

One reason this might happen would be if this particular mutation has a tendency to occur spontaneously. But the fact that it does not exist outside Vanuatu makes this unlikely. The obvious explanation, say the researchers, is that some paternal mtDNA enters the egg and recombines with the maternal mtDNA.

The second study, by John Maynard Smith and colleagues at the University of Sussex, focused on mutations in mitochondrial proteins from populations around the world. They found a large number of mtDNA sites where mutations occurred. But most of these mutations were found in only a limited geographic area. This suggests again that the mutations occurred very rarely and then spread locally by recombination of maternal and paternal mtDNA, say the Sussex researchers.

If recombination with paternal mtDNA is responsible for some of the variation in mtDNA, then its true mutation rate is much lower than biologists thought and Eve must have lived longer ago than the 100,000 to 200,000 years of current estimates. (Source: *New Scientist*, 13 March 1999)

Gene world's bad guys revamp their image

A genetic vandal, infamous for rampaging through the genome and destroying genes, may after all have

played a constructive part in evolution, a new report says.

Snippets of DNA called retrotransposons have a bad reputation. They reproduce by making RNA copies of themselves, which then turn back into DNA and slot into new positions in the genome. One particular retrotransposon, called *L1*, has been busy replicating itself in animal genomes for aeons, so that non-functional copies of *L1* now account for about 15 per cent of human DNA. Occasionally, such meandering has caused mutation and disease.

But *L1* may not be all bad, according to Haig Kazazian and his colleagues at the University of Pennsylvania in Philadelphia. They knew that *L1* RNA copies sometimes include neighbouring sequences. If these pieces could be large enough to include an active chunk of a gene called an exon, wandering *L1s* could copy the exon as well. Such "exon shuffling" is believed to have been a major driving force of evolution because it allows useful fragments of different genes to join up in new ways.

To test the idea, Kazazian and his colleagues took an exon containing a gene that confers antibiotic resistance and inserted it into human cells. The gene could become active only if the whole exon had hitchhiked a ride on an adjacent *L1* copy, and was then inserted correctly into an active gene. Cells in which this leap actually occurred would be made drug resistant.

The team found that about 1 per cent of transfers resulted in drug-resistant colonies. Since genes make up only 15 per cent of human chromosomes, this suggests *L1s* frequently managed to carry their exon cargo. (Source: *New Scientist*, 13 March 1999)

Jump to it

The first intact "jumping gene" in the human genome has been isolated by researchers in Germany.

RNA viruses can copy their genes into a host's DNA, creating DNA sequences known as retrotransposons. These fragments can reinsert themselves elsewhere in the genome, sometimes causing damage. This jumping eventually stops as the retrotransposons lose the genes vital for copying themselves. Eckart Meese and Jens Mayer of the University of the Saarland have found a retrotransposon on human chromosome 7 that still make complete viral proteins.

The researchers plan to test whether the fragment can really "jump". Meese suggests its genes could be beneficial—the proteins they code for may prevent infection by occupying sites where harmful viruses might bind. (Source: *New Scientist*, 6 March 1999)

Splitting hairs

Differences in hair structure could lead to a new screening test for breast cancer, say scientists in Australia.

A team led by Veronica James of the University of New South Wales in Sydney has found that the X-ray diffraction patterns of hairs from women with breast cancer are different from the patterns of hairs from healthy women. The same telltale pattern occurred in hair from women who did not have cancer but carried a mutation of the *BRCA1* gene known to increase the risk of contracting the disease.

The researchers believe the patterns—which arise from differences in the way molecules are organized within the hairs—reflect differences in the cell membrane as the hair develops in the follicle. (Source: *New Scientist*, 6 March 1999)

The panic gene

Men who suffer panic attacks may owe their disorder to the same gene mutation that turns the red eyes of a fruit fly white.

Fruit flies with white eyes have a defect in the *white* gene, which makes a protein that transports the amino acid tryptophan into the cells that make red eye pigments. Tryptophan is important to humans as well. We use it to make serotonin, a neurotransmitter that regulates mood.

Mood disorders are known to be linked to the region of chromosome 21 where *white* is located. So a team led by Akira Sano of Ehime University in Japan sequenced the *white* gene of 129 patients with mood or panic disorders as well as that of 130 healthy controls. They found that 75 per cent of the male patients had at least one copy of *white* in which a guanine base had been replaced by an adenine base. Only 50 per cent of male controls had a copy of this version.

Sano's study did not reveal any link between female mood disorders and *white*. He thinks it is important to find out whether the adenine version of *white* hinders the transport of tryptophan in people. If so, tryptophan supplements might be used to treat men susceptible to panic attacks. (Source: *New Scientist*, 6 March 1999)

Research on animal genes

Dutch firm claims commercial cloning success

A Dutch biotechnology company is claiming to have developed the first commercially significant application of nuclear transfer, the cloning technique used to generate Dolly the sheep.

Pharming, based in Leiden, says it has produced three female calf clones capable of secreting a human biopharmaceutical in their milk. These will eventually form part of a herd producing the drug in commercial quantities.

The calves were generated from cow foetus cells genetically engineered to express a human protein. The nuclei from these cells were fused with cow eggs that had the nucleus removed, then implanted into surrogate mothers. Pharming believes it is the first time that the technique has been used to create commercially useful transgenic animals.

Over the next year Pharming plans to generate an entire herd of the animals. These will be housed at a farm complex in DeForest, WI, home to Pharming's US research partner Infigen. Although the complex can accommodate up to 80 animals, Pharming will not say how many cows it intends to generate.

The identity of the protein is also being kept secret for commercial reasons. However, it is almost certain to be one of Pharming's three lead products. These are the human proteins lactoferrin, alpha-glucosidase and C1 esterase, which are used to treat a variety of infectious and genetic diseases.

Pharming has a long history of pioneering work in cow genetics. In 1988 it generated the world's first transgenic bull, and a decade later performed the first nuclear transfer on cows to produce two female calves. (Source: *Chemistry & Industry*, 15 February 1999)

Mouse "tricked" into producing egg from an African elephant

In an experiment that may help preserve endangered species or address human fertility problems, a mouse implanted with ovarian tissue from an African elephant successfully produced an elephant egg.

Purdue researcher John Critser, working with the Advanced Fertility Institute at Indianapolis' Methodist Hospital, transplanted frozen ovarian tissue from an African elephant into a mouse to see if it would produce an elephant oocyte.

The results from the experiment indicate that such transplanted tissue from a variety of female mammals may be used to regenerate cells for numerous species. Critser said that in theory, the elephant egg could be fertilized *in vitro* and then transplanted into a female elephant.

Procedures to do this and embryo transfer will require more investigation and development, he said.

This study was a follow-up to two others published by Critser in 1997 which demonstrated that frozen ovarian tissue from different species could produce viable eggs in immune-compromised mice.

In those experiments, cryopreserved ovarian tissue from mice or sheep were transplanted into mice. After hormone production was established, those mice with mouse egg transplants were mated and produced live pups.

The elephant tissues initially contained only a few immature follicles, but the mice were able to develop more mature follicles within 10 to 11 weeks, with one developing a mature egg two months after transplantation.

These findings indicate that storing ovarian tissue at low temperatures may provide a fairly simple way of preserving genetic material from female mammals, which has been difficult in the past.

He is now working with transgenic rats to develop further methods of transplanting frozen tissue into mice; he will then analyse the ovaries' ability to establish cycles and produce eggs.

The rat studies should also help improve techniques for embryo transfer, to be used in cloning. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 November 1998)

Hens with fly DNA laying foundation for future drug factories

Scottish geneticists have found a way to alter the DNA of chickens using genes from common fruit flies.

This bug-to-bird transfer opens the door to the creation of eggs that hold not just a white and a yolk, but proteins to fight human diseases. The potential exists to purify the drugs from the eggs or to simply eat the whole package.

One big advantage to chickens as pharmaceutical factories, said geneticist Helen Sang of the Roslin Institute, is that they are quicker and easier to raise than the sheep, goats and cows, currently being bred for that purpose.

In the past, geneticists have found chicken eggs difficult, said Sang. One problem is that chicken embryos are harder to reach than those of mammals. By the time they are neatly deposited outside the hen in an egg, the chick is relatively well developed, containing as many as 60,000 cells and past the point where scientists can easily insert a gene. So in earlier work, Sang's group developed a method in which they recovered the egg just after fertilization, added the foreign gene, and then incubated it in "host shells", which contain the egg white, critical to nourish the growing bird. But once they figured out how to inject the DNA, they then had to find a way to get it to incorporate itself into the chicken's genetic makeup, or genome, to create a transgenic chicken.

Sang said they found the answer in a gene called mariner, taken from the fruit fly, that pushes into the genome with a "simple cut and paste" mechanism.

This is not the first time a transgenic chicken has been created, but scientists say the fly gene method appears to be more efficient than other methods.

Poultry gene expert Bob Ivarie said, "This is a significant step forward." The use of the fly gene may improve the efficiency of altering chicken genes.

One problem with the method, however, is that there may be a "severe limitation" on the size of a gene that can be carried by the fly DNA. Sang said answering this question will be one of the next areas of research for her team. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 November 1998)

Improving gene transfer into livestock

About 10 years before they startled the world by cloning Dolly the sheep, scientists at The Roslin Institute south of Edinburgh had rocked the scientific community by producing the first healthy sheep carrying a human gene. Since then, a few research groups have used similar gene transfer techniques to build herds of sheep, cattle, goats, and pigs that make human proteins, often with the goal of milking them for valuable drugs. Now, a new method developed by a team of researchers in Wisconsin and California promises to make production of such transgenic livestock much easier than it is today.

Current gene transfer procedures for large animals are time-consuming and expensive, mainly because their efficiency is low.

Robert Bremel, formerly of the University of Wisconsin, Madison, and now managing director of Gala Design, a biotech firm in Sauk City, WI, his former Wisconsin student Anthony Chan, and their colleagues report that they have achieved a transformation efficiency approaching 100 per cent. They did this by introducing a foreign gene into cow eggs before they were fertilized rather than shortly after, as is currently done.

The increased efficiency should be welcome news to researchers who want to introduce genes into livestock, either to improve the strains or to use the animals to produce medically valuable proteins, such as monoclonal antibodies or vaccine proteins.

In the older techniques, researchers introduce a gene in a carrier, such as the DNA of a retrovirus that can insert itself into the host cell DNA, into an egg that has already been fertilized. But if the DNA does not insert until after the egg starts dividing, as is often the case, it ends up only in the descendants of the cell where it integrated, which might be a small minority of the total in the embryo.

To counter this problem, Bremel and his colleagues decided to use unfertilized bovine oocytes isolated in metaphase arrest, when the membrane that normally surrounds the nucleus is absent. The researchers reasoned that this would make it easier to get the foreign gene to the chromosomes so that it could integrate. In addition, putting the gene in the chromosomes of the egg itself would ensure that the gene would end up in all the cells, including the germ cells, of the animal produced when the egg was subsequently fertilized. In cows, "the DNA is incorporated better when inserted earlier", says Bremel.

The team first introduced the gene coding for the hepatitis B surface antigen into a retroviral carrier. They chose this gene, Bremel says, partly because the antigen makes an easily detected marker and partly because any transgenic animals could produce the antigen, which is used in hepatitis B vaccines. The researchers then injected the retrovirus into the oocytes, allowed them to mature, and fertilized them.

Of the 836 eggs injected, 174 developed into embryonic blastocysts, and 10 of these were implanted into five foster mothers. This yielded three pregnancies and four healthy calves, two males and two females, now about 2 years old. Tests on skin and blood cells revealed that all four animals carry the hepatitis B gene. In addition, the females secrete the antigen in their milk. And mating one of the bulls with a nontrangenic female produced twin offspring, both transgenic. The researchers say that this technique should work in other species, including primates, where immature egg cells can be manipulated during metaphase. Indeed, if the technique proves as efficient as it now appears, it might even make livestock cloning obsolete. (Source: *Science*, vol. 282, 27 November 1998)

Cloning made easy

The team that cloned Lady, the last surviving member of a rare breed of New Zealand cow, has now cloned no fewer than 10 identical calves from an adult Friesian. Born at the Ruakura Research Centre in Hamilton, New Zealand, in August and September, the calves show that cloning adult livestock is quickly becoming efficient.

David Wells of the Ruakura centre attributes the success to a culturing technique that creates a six-hour delay between fusing an adult cell with an egg stripped of its chromosomes and the start of cell division in the resulting embryo. This may give more time for the adult cell's chromosomes to be "reprogrammed". (Source: *New Scientist*, 14 November 1998)

Bubble wrap

The technique children use to blow bubbles has been borrowed by biologists to develop a method of freezing embryos that should work with a wide variety of animals.

Breeders use frozen embryos to preserve stocks of animals with valuable characteristics, but it works only with some species. In others, it kills the embryos. Rapid freezing is crucial. If it is too slow, ice crystals form inside cells and damage them. Speedy freezing also minimizes the time embryos spend immersed in freezing solutions, which are mildly toxic.

By developing specialized techniques, researchers have had success with some species that are notoriously difficult to freeze, such as pigs. Katrina Forest, Michelle Lane and their colleagues at the University of Wisconsin, Madison, hope that their simple method of protecting embryos will work for many different species.

Instead of putting the fertilized egg in a container of freezing solution, they take a tiny nylon loop and dip it into the solution. This suspends a thin layer of liquid across the loop, just as a child suspends a soap solution to blow bubbles. The researchers then place early embryos in this thin film and dunk it into liquid nitrogen. This freezes embryos almost instantaneously.

The researchers have tested the technique on one-cell hamster embryos, which are extremely sensitive to freezing. After thawing, the researchers compared the development of the frozen embryos with controls that were not chilled. Fifteen per cent of the thawed cells went on to form a ball of cells called a blastocyst, compared to 30 per cent of the controls. Previously frozen embryos that survived to the blastocyst stage were just as likely to develop to term as control embryos when implanted. No one has previously succeeded in getting hamster births from frozen embryos.

Lane, who now works at the Colorado Center for Reproductive Medicine in Denver, thinks the technique

might eventually be used for preserving human embryos. (Source: *New Scientist*, 23 January 1999)

Hopes of reviving old muscles

The gentle job of folding the delicate loops of our chromosomes into nuclei may fall to a surprisingly brawny protein, say fly geneticists in Baltimore.

The DNA in a single human cell would stretch to almost two metres if the molecules were unwound and laid end to end. But this filament must be packed into a nucleus only 10 micrometres across. Part of this organizational feat is carried out by proteins that help fold the DNA into the familiar, sausage-like shape of chromosomes.

Without proper packaging, chromosomes would tangle and tear each time cells divide. For that reason, many biologists expected that specialized proteins would dedicate themselves to the job of safeguarding genetic material. But now Cristina Machado and Deborah Andrew of Johns Hopkins University have found a DNA packing protein that seems to be moonlighting.

The team hunted for new packing proteins by looking for antibodies that bind to proteins all over the surfaces of chromosomes. They found one in the fruit fly *Drosophila melanogaster*, and in human cells. Oddly, the same antibody also binds to a protein in the muscle tissue of fruit fly embryos.

When the team isolated a piece of the fly gene that codes for their packing protein, the link to muscle strengthened. That chunk resembled part of the gene for vertebrate titin, a known muscle protein. Titin is a stretchy giant among proteins: a single molecule can stretch more than a micrometre. In muscle, it helps absorb shocks and prevents muscles overstretching.

The researchers say they now have direct evidence of titin's strength and organizational skills. They used standard techniques to cripple the titin gene, which they call *D-Titin*, in flies. These insects had disorganized, ineffectual muscles, suggesting *D-Titin* is indeed the insect version of the vertebrate muscle gene.

And the flies' DNA was not in much better shape. Many of their cells had broken chromosomes and their nuclei had swollen to five times the normal size to contain the bulging DNA. The researchers picture titin as organizing long sections of chromosomes into a compact structure. Now they want to confirm that suspicion by using electron microscopy to examine exactly how the protein is woven into each chromosome. (Source: *New Scientist*, 2 January 1999)

Signal transduction gets hairy

Efforts to elucidate an important developmental signalling pathway have opened the door to a broad range of biotechnology applications—and generated an extremely hairy mouse. The mouse, which expresses a truncated, constitutively active form of the beta-catenin signalling protein in its skin cells, generates new hair follicles as an adult. Ordinarily, new follicle formation stops before birth. While the discovery received extensive popular coverage as a potential cure for baldness, the researchers emphasize that substantial barriers remain to such an application. "Whether such technology would be advantageous in a clinical setting would be dependent upon whether animal skin could be induced to make hair follicles without progressing to skin tumours", explains Elaine Fuchs, a professor in the Howard Hughes Medical Institute at the University of Chicago and senior author on the study. While the tumours appear late in the animal's life and are relatively benign, they are potentially disfiguring. Fuchs suggests that a more imminent application might be the production of transgenic sheep which yield more wool. The team is currently trying to develop a more detailed model of the signalling pathway, which may also be involved in the development of teeth, nails, mammary glands, and other structures. (Source: *Nature Biotechnology*, vol. 17, January 1999)

Oocyte gene transfer

Taking advantage of what may be an evolutionarily-conserved "back door" genomic modification, researchers at Gala Design (Sauk City, WI), the University of Wisconsin, Madison, and the University of California, San Diego have developed a technique that may have extensive applications in generating transgenic animals. During the MII meiotic arrest in oocyte development, the nuclear envelope breaks down. Reasoning that this event would allow access to the genome, the scientists infected bovine MII oocytes with a pseudotyped recombinant retrovirus carrying a reporter gene. The virus integrated efficiently, and embryos derived from the infected oocytes carried the gene in all cells. Robert Bremel, managing director of Gala Design and senior author on the work, argues that the permeability of arrested oocytes to gene insertion may have played a role in mammalian evolution, allowing naturally occurring retroviruses or transposons to deliver useful traits: "Operating repeatedly through many generations, this system would provide a mechanism for periodic, dramatic quantum changes in phenotype". Bremel adds that the pseudotyped retrovirus allows researchers to take control of this process for a broad range of species, since "these vectors are virtually universal gene transfer entities". The company plans to focus initially on expressing novel proteins in transgenic cows' milk. (Source: *Nature Biotechnology*, vol. 17, January 1999)

After eight years, researchers break genetic code of worm

In a scientific first that some geneticists are comparing to man's first walk on the moon, a US-British team deciphered the genetic code of the first complete animal, *Caenorhabditis elegans* (*C. elegans*).

It took the team eight years to sequence *C. elegans*, a lowly worm that lives in the soil and in rotting plants.

Although *C. elegans* is only about one millimetre long, it shares many traits with humans, making it important for carrying out studies on early development, neurobiology and ageing that parallel human biology.

Another contribution the international research team made was to establish successful methods for sequencing complex genomes, as well as computers and software to automate the task, which led the Human Genome Project to accelerate completion of the human genome sequence by two years. The new target date is 2003.

C. elegans has 97 million base pairs of DNA, and its genetic material is packed on six chromosomes. Previously, the largest genome fully sequenced was fungus, which has about 12.5 million base pairs. By comparison, humans have 3 billion base pairs.

About 19,099 genes in *C. elegans* can code protein, an important feature of animals. Some 40 per cent of those genes match genes in other organisms, including humans, while 74 per cent of the known human genes have counterparts in *C. elegans*.

C. elegans is one of a group of animals that are shedding light on biology in general and human function in particular. Others include yeast and frogs, which reveal information on cell division, and Drosophila and *C. elegans*, which provide information on cell proliferation. *C. elegans* is also being studied to learn about apoptosis, or programmed cell death.

One unexpected result of the research was the high number of gene clusters, or families of similar genes located in clusters on the same chromosomes. There were 402 clusters found in *C. elegans*, many of them novel genes. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 21 December 1998)

Cat virus yields clues on hard-to-treat HIV

The virus that causes feline AIDS has helped researchers to develop a potential treatment for drug-resistant HIV. The new chemical, which is active against the feline immunodeficiency virus, was found to work well against the human virus in test-tube experiments.

Drug-resistant HIV shrinks the site where inhibitor drugs bind to the protease enzymes that replicate the virus. Chi-Huey Wong and his colleagues at Scripps Research Institute in La Jolla, CA, noticed that the feline equivalent of HIV uses a protease with a naturally small binding site, similar to drug-resistant HIV. "That enabled us to use feline protease to develop a new drug", says Wong, who will report the work in the *Journal of the American Chemical Society*. (Source: *New Scientist*, 13 February 1999)

Fish tell tale of environmental toxins through gene mutations

Tiny "reporter" fish have been created through genetic engineering to determine if chemicals caused DNA mutations.

The fish are allowed to swim in an environment in which a chemical is dissolved. After an appropriate time in the polluted environment, the fish is retrieved and its DNA is examined to determine if mutations have occurred.

These transgenic fish could be used to rapidly test some of the 2,000 new chemicals—pesticides to fragrances—introduced into the environment each year.

At the annual meeting of the American Association for the Advancement of Science in Anaheim, CA, Richard Winn, assistant research scientist at the University of Georgia, Athens, said the fish could replace current tests that are less specific or more expensive.

"The transgenic fish carry specific DNA sequences that serve as targets for mutations within a bacteriophage or plasmid vector", Winn said. Because the fish swims in the chemical of interest, virtually all the animal's cells are exposed to the chemical, allowing for more opportunity to test the potency of the chemical to cause mutations.

The genetic damage noted can be associated with development of diseases such as cancer.

"Among the benefits offered by transgenic animal models is their promise for significantly improving current methods used to evaluate the genetic health risks posed by exposure to contaminants in the environment", Winn said.

Scientists created the inch-long fish, a species of the Southeast Asian medaka, by inserting a specific gene into the fish's DNA. The fish incorporates the DNA into its own genetic fingerprint and then passes that gene to subsequent generations. Winn said this key part of the transgenic equation—creating new generations of transgenic animals—has been accomplished.

The fish is then placed into an aquarium and the chemical is added to the water.

"The idea of having an animal swimming in the chemical so that all its tissues are exposed to the chemical is superior to feeding chemicals to transgenic mouse models", Winn said.

One of the major advantage so fish over current mouse models is that the fish are far easier and far less expensive to maintain than colonies of mice.

After exposure, the fish "reports" back. It is retrieved, sacrificed and its DNA is extracted from its tissues. The DNA is compared to the original DNA strand.

Preliminary tests show that the reporting technique works efficiently. Winn also said the naturally-occurring mutations are similar to those seen in mouse models, meaning that the fish will be useful as an agent to detect these changes. He said the fish have the potential to discover not only toxic effects of chemicals, but also synergistic and additive effects. (Source: *McGraw Hill's Biotechnology Newswatch*, 1 February 1999)

All for one and one for all

A single gene may guide individuals within colonies of tiny animals called hydroids to develop in radically different ways, say researchers. Hydroids, which are relatives of hydra, take the form of polyps, and have a base at one end and tentacles at the other.

Individual hydroids in some species are highly specialized. Some members of a colony do all the eating,

others reproduce and some are little more than a single stinging tentacle.

Paulyn Cartwright and her colleagues at Yale University monitored the activity of a gene called *Cnox-2* that defines the head-to-tail axis of the body as polyps develop. They found that feeding polyps have lots of the gene's protein product near their base, but little up near the tentacles. Defensive polyps, which have pronounced tentacles, have little *Cnox-2* anywhere.

When Cartwright removed all the feeding polyps from a colony, she found that some defensive polyps changed into the feeding form. In the transformed polyps, *Cnox-2* became more abundant near their base.

The study is the first to show that a single gene can dictate much of the specialization in colonial animals, says Mark Martindale of the University of Hawaii's Kewalo Marine Laboratory near Honolulu. (Source: *New Scientist*, 13 March 1999)

Take heart

The discovery that mouse bone marrow cells can be turned into heart cells could lead to new treatments for human heart disease, say Japanese scientists.

Heart cells do not regenerate, so doctors would like to have a ready source of replacement for heart tissue that is killed. Keiichi Fukuda of the Keio University School of Medicine in Tokyo has found that treating mouse bone marrow stromal cells with a chemical called 5-azacytidine can turn them into heart muscle cells.

Researchers already knew the chemical could make certain embryonic cancer cells differentiate into heart cells. Now doctors hope they will be able to treat patients with newly formed heart cells created by manipulating their own bone marrow. (Source: *New Scientist*, 6 March 1999)

Reutilization of silkworm urea for amino acid synthesis

The National Institute of Sericultural and Entomological Science (NISES) of the Ministry of Agriculture, Forestry and Fisheries has discovered that silkworms can reutilize their urea effluent as a source of nutrition by utilizing an enzyme synthesized in mulberry leaves, synthesizing amino acids from urea for use as the raw material for producing silk thread. The institute confirmed that silkworms cultured with mulberry leaves are reutilizing the urea that they generate as a nitrogenous excretion, but that silkworms cultured with artificial feeds are not reutilizing the urea. Organisms capable of utilizing urea as their source of nutrition are quite rare, so the research institute plans to develop an artificial feed of high nutrition value for silkworms.

Silkworms generate urea in their bodies, but with silkworms subsisting on mulberry leaves, the urea is dissolved into ammonia inside their digestive system prior to being excreted, then the urea is converted into amino acids after reabsorption in the form of ammonia. In this case, the mulberry leaf contains the enzymatic urease (that decomposes urea into ammonia), which continues to function without being assimilated in the silkworm digestive system. The silkworm absorbs this ammonia and synthesizes glutamic acid, an amino acid, which is utilized as the raw material for producing silk thread protein. Therefore, the mulberry is not serving simply as a source of nutrition for silkworms but also as a part of the biological function relating to metabolism and growth in the silkworm.

This function is not present with artificial feeds which are produced by heating and processing, and is a highly interesting phenomenon from the aspect of food function research. Further details from: National Institute of Sericultural and Entomological Science (NISES), Public Relations Dept., 1-2, Owashi, Tsukuba City, Ibaraki Pref. 305-0851. Tel.: +81-298-38-6026. Fax: +81-298-38-6028. (Source: *JETRO*, February 1999)

Three-legged chickens reveal how limbs develop

Biologists have been trying to work out the details of limb development for at least ten years, and have discovered several genes that build arms or wings and legs. But all these genes operate in both structures. What has been missing is an explanation of how the obvious differences arise.

In the past few years, several research teams have discovered a few genes that are selectively expressed: *Pitx1* and *Tbx4* in legs, and *Tbx5* in arms or wings. Now, in two independent reports, Cliff Tabin of Harvard Medical School in Boston and his colleague Malcolm Logan and another team led by Michael Rosenfeld at the University of California, San Diego, and Juan Carlos Izpisúa-Belmonte at the Salk Institute in La Jolla have shown that *Pitx1* plays a key role in orchestrating the development of legs.

Both research teams used modified viruses to carry copies of the genes into one developing wing of chicken embryos. They found that *Pitx1* was able to switch on the activity of the other leg-specific gene, *Tbx4*. With both genes functioning, the entire limb muscle structure became more like that of a drumstick, with a claw and four digits at the end: normal chickens have four toes, while their wings have the equivalent of three fingers. The transformed limb also lacked feathers. The wing on the other side of the embryo, which had not received the dose of *Pitx1*, developed normally.

The team in California also disrupted the *Pitx1* gene in mice. In this case, the animals' rear legs developed smaller, thinner bones, similar to those in their upper limbs. Intriguingly, these "knockout" mice also had undersized palates and lower jaws, two other regions where *Pitx1* is normally expressed. Rosenfeld says this suggests the gene might act in leg development by encouraging the growth of bones and muscles at crucial moments.

Pitx1 may not be the whole story, however. Even when it was present, the developing chicken wings kept some of their normal bone structure, and the activity of the wing-specific *Tbx5* gene was unchanged (*Genes and Development*, vol. 13, p. 484; *Science*, vol. 283, p. 1736).

Tabin says the challenge is now to understand how these genes and others turn the same theme of muscle, bone, joint and digit into two dramatically different structures. (Source: *New Scientist*, 20 March 1999)

Mammals may keep their unique identity

The sudden failure of a clever trick of inheritance may explain why certain cloning experiments fail, a new report suggests. It may also be behind the creation of some mammalian species.

A basic rule of heredity is that animals inherit two copies of every gene, one from each parent. In mammals, however, there is a strange twist to this rule known as imprinting. A small percentage of mammalian genes are biochemically marked or "imprinted" so that only the copy from one parent is switched on. But Shirley Tilghman and her colleagues at Princeton University in New Jersey have found that this mechanism can go wrong. When they mated two closely related species of mouse, *Peromyscus polionotus* and *P. maniculatus*, they found that both copies of some imprinted genes were turned on in offspring, making them develop abnormally.

Even more bizarrely, which imprinted genes were affected depended on which species contributed sperm and which the egg. When *P. maniculatus* served as the father, the hybrid foetuses were oversized and rarely survived. When the same species was the mother, the offspring were 40 per cent smaller than normal, and would probably not survive long in the wild.

One possible explanation for the results is that "imprinting recognition proteins" in the egg of one species cannot read all the imprinting marks on the DNA of another. The researchers say this incompatibility may have contributed to mammalian evolution, since reproductive isolation is one characteristic of species that have diverged. "This inability of one species to recognize the imprinting of another appears to create a strong reproductive barrier between them", Tilghman says.

She believes that the mechanism could also explain why some attempts to clone animals have failed to produce viable embryos. In these experiments, the cell of one species is fused with a cow egg that has been stripped of its own nuclear genetic material. Scientists are interested in using this technique to clone rare animals from which it is difficult to obtain eggs. (*New Scientist*, 12 December 1998)

Research on plant genes

Antibody tobacco emerging as hero in war on cavities

Tobacco, the plant world villain blamed for lung cancer and heart disease, could become the first long-term method of preventing tooth decay, with a little help from genetic engineering.

Planet Biotechnology, Inc. has launched Phase I/II trials of CaroRX™, a monoclonal antibody produced from genetically transformed tobacco plants.

CaroRX™ has been shown to provide long-term protection from the *Streptococcus mutans* bacteria, the primary cause of tooth decay. CaroRX™ is a type of antibody called secretory immunoglobulin A, or a SigA, which is naturally produced by the body to protect mucus areas in the mouth and other parts of the body from harmful bacteria.

CaroRX™ combines with and prevents the bacteria from reacting with the tooth. The end result is that other bacteria, not responsible for tooth decay, "grow in its place", removing the niche originally occupied by *S. mutans*. Based on current research, CaroRX™ has been shown to "eliminate the presence of bacteria for long periods of time". The previous study covered an 18-month period and "all of the treated patients have had long-term removal of these bacteria". (Source: *McGraw Hill's Biotechnology Newswatch*, 16 November 1998)

Promiscuous junk DNA has invaded thousands of plant species

Plants that do not interbreed can still exchange DNA using go-betweens such as fungi, viruses or aphids, new research suggests. The findings are likely to be seized on by opponents of genetic engineering, who fear the spread of modified genes from crops to wild plants. But the scientists behind the research stress that such DNA transfers are very rare events.

Jeff Palmer and his colleagues at Indiana University in Bloomington have discovered a stowaway gene segment in scores of unrelated flowering plants, including coffee, bananas, cucumbers, periwinkles and foxgloves. They speculate that the segment originated in fungi, as fungal species including baker's yeast (*Saccharomyces cerevisiae*) are known to carry it. "We think there was at least one original donation from a fungus to a plant", says Palmer. Since then, it may have been shuttled from plant to plant by aphids or viruses.

Palmer's group screened 335 families of flowering plants for the segment, a chunk of DNA called an intron. Introns are junk DNA that is clipped out of genes before they are transcribed into proteins. The stowaway intron, which buries itself in the same gene whichever plant it invades, appeared in 48 of the families.

Through analysis of plant family trees, Palmer and his colleagues have found that in 32 of these 48 cases, the intron spread "laterally" between unrelated species rather than "vertically" through inheritance. Scaling up to the 13,000 known families of flowering plants, Palmer says the intron must have jumped species at least 1,000 times.

The promiscuous intron studied by Palmer carries a molecular tool for jemmying itself into DNA. This tool, the gene for an enzyme called an endonuclease, always wedges the intron into *cox1*, a gene vital for energy metabolism in plants.

Sequences lacking endonuclease genes are probably much less mobile, says Palmer. And even with its

endonuclease, the stowaway intron has probably jumped species perhaps just once every 5 million years.

Given the limits to lateral DNA transfer, Palmer does not think that the risks posed by genetically engineered plants need to be reassessed in the light of his research. (Source: *New Scientist*, 28 November 1998)

Blue for danger

The botanical equivalent of a Geiger counter has been brought to life by scientists in Switzerland and the Ukraine. The team has engineered a plant that warns of dangerous levels of radiation.

Currently the only way to see if plants have been exposed to dangerous levels of radiation is to look for cell damage under the microscope. But this is labour-intensive, and the damage can be easy to miss.

Now a team led by Barbara Hohn at the Friedrich Miescher Institute in Basel has found a simpler way. They added a gene from the bacterium *Escherichia coli* to thale cress (*Arabidopsis thaliana*). The *E. coli* gene itself contained a foreign piece of DNA that stopped it producing an enzyme.

The idea was that if the plants were exposed to high-energy radiation, mutations would occur, some of which might knock the foreign DNA out of the bacterial gene. The two parts of the *E. coli* gene could then join up, restoring its function. If the plant tissue was then mixed with a stain that turns the bacterial enzyme blue, the mutated parts would be obvious.

Sure enough, when exposed to radiation then stained, parts of the plants turned blue. The researchers say there is a clear relationship between the radiation dose and the plants' colour—the more radiation, the bluer the plant.

The plants have also been tested near the Chernobyl nuclear reactor that exploded in 1986. The modified plants accurately reflected the levels of radiation in the environment. (Source: *New Scientist*, 28 November 1998)

Thinning ozone could awake jumping genes in plants

The idea of ultraviolet radiation seeping through Earth's depleted ozone shield and damaging the DNA in organisms is a familiar one. But rising UV levels may also reactivate destructive "jumping genes" in maize and other plants. The resulting surge in genetic mutations could threaten the quality of grain supplies, warns a molecular geneticist in California.

Jumping genes, or transposons, are bits of DNA that can snip themselves out of their existing position on a chromosome and insinuate themselves elsewhere, disrupting the section of DNA they happen to land in. Host cells have evolved ways to stop transposons from jumping. But today, about half the maize genome consists of inactive transposons and other mobile elements, so the potential for disruption is great.

Scientists already know that massive doses of UV radiation will rouse one transposon, known as *Mutator*, from its torpor to begin jumping again. Virginia Walbot of Stanford University found that much smaller doses of UV-B radiation could do the same trick. Walbot exposed corn pollen with UV-B radiation of the same intensity they would experience under 33 per cent ozone depletion—a lower level than that already observed at high latitudes. Walbot used a marker gene that causes spotted kernels when *Mutator* is active. She found that 6.2 per cent of the seeds fertilized by the irradiated pollen showed *Mutator* activation. But there was no evidence of *Mutator* activity in the progeny of untreated pollen.

If transposons are also reactivated in nature, this should not affect corn yields directly, because each pollen mutation affects only a single seed, and most modern farmers buy fresh seed each year. But over time the mutations could accumulate in seed supplies.

Similar transposons occur in many wild plant species, raising the possibility that ozone depletion could hit natural ecosystems in a similar way. (Source: *New Scientist*, 6 February 1999)

Altering enzymes

Scientists at the United States Department of Energy's Brookhaven National Laboratory and the Carnegie Institute of California, have managed for the first time to change the properties of a plant enzyme. Although these kinds of events are commonplace in microbial or mammalian cells, it represents a major breakthrough in plant biotechnology.

"We have shown that it is possible to change an enzyme's function dramatically by tweaking its structure just slightly", said John Shanklin, who co-led the research team. Shanklin and his colleagues worked with enzymes called desaturases and hydroxylases.

Shanklin and his colleagues have studied desaturase and other plant enzymes for several years. In 1997, a team from Brookhaven and Sweden's Karolinska Institute were the first to alter a desaturase so that it made fatty acids bend at a different point and created an oil with different characteristics. (Source: *European Chemical News*, 30 November-6 December 1998)

Substances from holly to treat diseases

Over the past 18 months chemists in Ireland have extracted several compounds from the European holly bush that could one day treat diseases such as cancer.

Holly extracts have been prescribed as folk remedies in Europe for centuries to treat everything from dizziness and hypertension to cancer. "These are still used in parts of Spain and Turkey", says Myles Keogh at the Galway-Mayo Institute of Technology in Galway.

Although local holly varieties are under scrutiny by chemists in China and Japan, the properties of the European holly bush (*Ilex aquifolium*) had been ignored by researchers, so Keogh and his colleagues decided to investigate.

The team has now isolated several potential medicinal chemicals. Although their potency has yet to be tested, Keogh hopes some might be valuable because other substances from the same chemical families have found a place in medicine.

From holly-bush roots Keogh has isolated three saponins. Similar compounds in soya beans and yams have been linked with resistance to cancer, while others have been tested as adjuvants that strengthen the immunological effects of vaccines. Holly's saponins are slightly unusual in that they each carry a pair of sugars. "The sugars make them more permeable, so they might get into cells more easily", says Keogh.

More recently, the team has isolated compounds called triterpenes from the bush's bark. They are already under evaluation for medicinal properties. Betulinic acid, a triterpene from birch bark, is being tested against skin cancer.

Keogh says the triterpenes in holly are tethered to fatty acids, and are unusually abundant. They make up about 20 per cent of the bark's weight, so if the compounds turn out to be useful it may be possible to extract enough triterpenes for commercial use. (Source: *New Scientist*, 19/26 December 1998 to 2 January 1999)

Photosynthetic Harvest designs tobacco that exudes drugs from roots

Researchers from Photosynthetic Harvest Inc., a biotech company based in Willingboro, NJ, have genetically engineered tobacco plants to produce a variety of human and microbial proteins, promising a cost effective method of production.

Ira Pastor, Director of business development for Photosynthetic Harvest Inc. said that the protein development segment of the worldwide pharmaceutical market equals some $20 billion annually. Recombinant proteins are currently produced in bacteria. The problem with this is the high cost of purification.

Photosynthetic Harvest Inc.'s methods rely on keeping their plants alive to produce high yields over the long run, said Pastor.

The technology that Photosynthetic Harvest Inc. has developed, known by its proprietary name of Rhizosecretion, is more efficient because the same plants can be continually "milked" of their valuable harvest. The company describes Rhizosecretion as a technology that "takes advantage of the ability of a green plant to continuously secrete (exude) large quantities of organic compounds and proteins from its roots into a simple aqueous medium". Training the plants to secrete proteins into water will simplify purification and retrieval of target molecules.

"We are optimizing a molecular farming system which is able to continuously secrete recombinant proteins, without the need to destroy the actual plant material", Pastor said. "We keep the plant alive and create a mini 'biofactory' for the continual production of novel chemicals".

Pastor believes that all the life science corporations may one day benefit from this technology, but, ultimately, individuals with specific medical needs will have the most to gain. (Source: *McGraw Hill's Biotechnology Newswatch*, 18 January 1999)

Rare plants may stop the body from sabotaging chemotherapy

One of the body's greatest blunders in the fight against cancer is to repair tumour cells that would otherwise succumb to chemotherapy. Now chemists in Virginia have discovered plant extracts that could deliver a killer blow to wounded cancer cells by blocking such repairs.

Sidney Hecht and his colleagues in the departments of chemistry and biology at the University of Virginia in Charlottesville screened extracts from several plants to see if they could block the action of DNA polymerase. This is the main enzyme that repairs damage inflicted on cancer cells' DNA by bleomycin, cisplatin and neocarzinostatin, chemotherapy's "heavy guns".

Hecht discovered five substances in the roots of a rare Californian plant, *Schoepfia californica*, that are capable of blocking the action of DNA polymerase. The most potent inhibitor was anacardic acid, a compound related to salicylic acid, the active component in aspirin. When these were combined with bleomycin, the mixture killed cultures of cells twice as effectively as bleomycin alone. (Source: *New Scientist*, 9 January 1999)

Pondweed picked for protein production

Common pondweed could become the protein factory of the 21st century. A US researcher has developed the first procedure to genetically engineer duckweek to produce pharmaceutical proteins and industrial enzymes.

Anne-Marie Stomp of North Carolina State University believes her gene expression and protein production techniques are potentially less expensive, more productive and less risky than existing ones.

Stomp became interested in duckweed's protein-producing potential while studying wastewater remediation. She became intrigued with the weed's natural ability to suck up nutrients, and learned that it was already being used to treat wastewater.

The protein-rich weed—made up of many tiny disc-shaped plants—can double in size within a day by cloning itself. It can also grow in a variety of environments, including stainless steel vessels, greenhouse pools or wastewater treatment ponds, Stomp says. This flexibility means it can be used for pharmaceutical manufacture, or large-scale industrial enzyme production.

Research will initially focus on producing therapeutic proteins in engineered duckweed. The system will have one major advantage over rival techniques that use mammalian cells or transgenic animals: "Because plants don't transmit human viruses, our system avoids the risk of contamination by infectious agents", Stomp explains.

The market for pharmaceutical proteins such as insulin or α-interferon is worth over $10 bn/a, Stomp estimates. Patents on many of these drugs are nearing expiry, and industry is searching for new and cheaper production techniques. Novel production processes for industrial enzymes are also much in demand. Duckweed technology could even transform wastewater from an

economic liability to a productive asset, Stomp predicts. (Source: *Chemistry & Industry*, 21 December 1998)

Silencing gene silence

Plant biotechnologists have a love-hate relationship with the phenomenon known as posttranscriptional gene silencing (PTGS), a mechanism by which plant cells shut down the expression of highly abundant transcripts. PTGS appears to have evolved as a defence against viruses, raising the possibility that plants could be "immunized" with viral transgenes. At the same time, the phenomenon often prevents high-level expression of desirable transgenes. Researchers at Washington State University (Pullman, WA) reported the discovery of a viral protein that shuts off PTGS. Cells expressing the P1/HC-Pro gene from tobacco etch virus permit high-level expression of a reporter gene that would ordinarily be silenced. While the viral gene would presumably facilitate the expression of transgenes in plants, James Carrington, a professor in the Institute of Biological Chemistry at Washington State and senior author on the study, warns that silencing is still a double-edged sword: "A shutdown of gene silencing would likely enhance susceptibility to most viruses. This could potentially be a problem in large-scale outdoor field releases." Nonetheless, several companies are exploring the possibility of using P1/HC-Pro to suppress PTGS, and Carrington hopes that the finding will aid in uncovering the underlying mechanism of the phenomenon. (Source: *Nature Biotechnology*, vol. 17 January 1999)

Plant polyketides

Using reverse genetics and biochemistry, a German-Finnish collaboration headed by Joachim Shroeder and Teemu Teeri have unmasked a role for a member of the chalcone synthase (CHS) family, gchs2, which suggests that CHS-related enzymes are involved in the biosynthesis of a much larger range of plant products than previously realized. The CHS superfamily synthesizes pyrones by using various substrates in a three-step condensation reaction of the polyketide pathway. Deploying an antisense version of a cDNA from a CHS-like protein from the ornamental plant *Gerbera hybrida*, the team was able to phenocopy a null mutation in gchs2. They then used nuclear magnetic resonance and mass spectroscopy to compare products of secondary metabolic pathways in wild type and "mutant" plants, identifying two compounds whose biosynthesis was not known to originate from the polyketide pathway. These compounds also have known biological activities—they confer insect and pathogen resistance. (Source: *Nature Biotechnology*, vol. 17, January 1999)

DNA switch may yield super foods with healing doses of vitamin E

The scientists from Reno's University of Nevada found a way to boost plant production of the most potent form of vitamin E, a nutrient that has been shown to protect against degenerative diseases but only if people eat enough of it, said biochemist Dean DellaPenna.

The problem, he said, is that few people meet the recommended daily requirements for vitamin E, which is 10 to 15 international units a day, because the richest sources—like sunflower oil—are not commonly found on the dinner table.

Mega doses—10 to 40 times above the RDA—that have been shown to prevent diseases like heart attacks and cancer, are impossible to achieve without taking supplements, which many people cannot afford, he said.

One solution is to boost vitamin E production in plants that do not make much of it.

The Nevada research team did just that by tinkering with the DNA that controls metabolism in Arabidopsis. DellaPenna, who led the research team, said vitamin E come in four forms, but the alpha form is far more nutritious than the best of the other three, the gamma form. So the scientists inserted a gene for an enzyme that converts the gamma to alpha form of vitamin E, leading to a nine-fold increase in the desired vitamin.

Large seed companies are working with the team to make the change in common foods, like corn and soybeans. DellaPenna said other groups are taking a genetic approach to increasing key nutrients in plants, such as folates and beta carotene. (Source: *McGraw Hill's Biotechnology Newswatch*, 21 December 1998)

Algae cousins look the same, but differ in cancer fighting powers

Just as twins have different personalities, researchers have found that look-alike plants of the same species produce compounds that differ widely.

For example, one form of blue-green algae—that actually looks red—growing in a mangrove off the southern coast of Curaçao makes a product that is showing promise in the treatment of cancer.

But 160 feet away, another collection of the exact same algae does not produce the anticancer compound.

Researchers reporting the differences at the annual National Sea Grant College Program symposium held in conjunction with the meeting of the American Association for the Advancement of Science in Anaheim, CA, said the differences in populations of seemingly identical species means developers of beach and other resorts or businesses have to be extra certain the projects do not destroy medical gold mines.

In fact, William Gerwick, professor of medicinal and natural products chemistry at Oregon State University, Corvallis, said his protests and those of other international scientists succeeded in halting development at a critical site on Curaçao, the Caribbean island that is part of the Netherlands Antilles. The Barbara Beach area on the south side of Curaçao is rich with microalgae growth amid the island's mangroves.

One collection of the algae, *Lyngbya majuscula*, produces Curacin A. Gerwick said Curacin A has shown curative ability in fighting small cell lung cancers in

animal models. He said he is working with Novartis Pharmaceuticals to test the product in cancer patients.

Just 50 metres to the south, Gerwick said, there is another collection of *Lyngbya majuscula* that does not produce Curacin.

Lyngbya can be found amid mangrove trees all along the Curaçao coast and various collections produce different chemicals, several of which may prove to be useful human medications, Gerwick said.

Remarkably, Gerwick said, that when a Curacin A-producing *Lyngbya* is grown in the laboratory, it still produces the anticancer compound. He speculated that something in the environment ignited a genetic signal that caused production of Curacin A, while other factors cause production of different chemicals.

Gerwick and colleagues are attempting to isolate the genetic differences between the "races" of *Lyngbya*. He said the discovery of these differing "chemotypes" among plants means decisions about disturbing natural habitats have to be closely considered to "preserve and hopefully capture the useful biosynthetic capabilities of these life forms".

Gerwick said the Curaçao story is just one example of how similar-looking organisms may produce a wide variety of useful or toxic substances.

Several promising drugs have been developed from algae, even though, Gerwick said, algae are best known for production of toxins that adversely affect human and animal health by poisoning fish and shellfish. (Source: *McGraw Hill's Biotechnology Newswatch*, 1 February 1999)

Research on viral genes

AIDS drug breaks up "fatal handshake" between HIV and cells

A new drug to fight AIDS takes a novel approach to the disease, breaking up the "fatal handshake" between the killer virus and its target, the cells of the human immune system.

In preliminary research, a team from the University of Alabama at Birmingham treated patients with the new drug, called T-20, that fights HIV-1 in a manner that is different from the drugs used in the potent cocktails that are now saving lives.

The doctors treated 16 patients, in groups of four, with varying doses of T-20 for two weeks. The amount of virus in the blood dropped to undetectable levels in four patients getting the highest doses of T-20, which was injected twice a day, said Michael Saag, a UAB professor of medicine. Saag says they saw a "100-fold" drop in viral load during the study, which is comparable to the hottest new anti-AIDS drugs—the protease inhibitors.

Scientists believe that T-20—a synthetic peptide—will probably be used first in patients who have developed resistance to current therapies or in those who are not helped at all by today's drugs.

"This is the proof of concept of a third major avenue of attack", said Saag.

Currently available AIDS drugs, such as AZT, 3TC and the protease inhibitors, are aimed at enzymes that allow HIV-1, the virus that causes the deadly disease, to replicate in a patient's body. The new drug fights AIDS in a different way, by preventing a process called fusion in which the virus tucks itself into the cell.

Chemist M. Ross Johnson said, "The fusion mechanism is the central thing, the fatal handshake."

Johnson, who heads Trimeris and was one of the authors of the paper, said that currently available anti-AIDS drugs work after the infection has occurred. T-20 prevents infection.

HIV-1 uses a molecule called gp41 to perform this fusion process. T-20 jams this molecule, leaving the virus floating aimlessly about until it dies, said Saag.

The current trial is the first to show that this method can work in living AIDS patients.

No side effects were seen during the study, although Saag admits that patients only received the drug for 10 to 14 days, which may have been too short a time for them to appear.

One drawback to the drug, Saag said, is that it cannot be packed in a pill.

Patients would have to give themselves under-the-skin injections of T-20. For that reason, he says, "it is unlikely to be a first line drug", he said.

But, he said the study shows, for the first time that interfering with the gp41 molecule can lock out the virus. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 November 1998)

Can IL-2 smoke out HIV reservoirs?

Potent cocktails of anti-HIV drugs have been enormously successful in keeping AIDS at bay in HIV-infected people. But although these combination therapies can knock the virus back to undetectable levels in patients' blood, HIV continues to lurk in "reservoirs"—cells that harbour the virus where antivirals cannot get at it. Now, new studies by a team at the National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, MD, indicate that a natural immune system regulatory molecule called interleukin-2 (IL-2), if given to patients along with combination therapy, can flush HIV from at least one reservoir out into the open. The finding raises hope that it may one day be possible to rid people of HIV entirely. "It's a courageous approach and the results are very intriguing", says immunologist Robert Siliciano at the Johns Hopkins Medical Center in Baltimore.

One known HIV reservoir is in T-cells—immune cells that are HIVs primary target. When infected T-cells are active, any HIV they harbour is also active and begins to replicate, making it open to attack by combination therapy. But T-cells also have a quiescent state, during which their latent cargo of HIV is dormant and invisible to antiviral drugs for years at a time. Because IL-2 has a potent ability to activate a number of immune cells, including T-cells, NIAID director Anthony Fauci and his colleagues decided to give patients IL-2 to see if it would

wake up their resting T-cells and the HIV they contain and make it vulnerable to attack.

Fauci reported that the NIAID team studied a group of 26 HIV-infected patients: 12 received a combination of at least three antiretroviral drugs for one to three years and 14 received similar combination therapy plus IL-2, given repeatedly but with a minimum of eight weeks between treatments. After treatment, all 26 had undetectable levels of HIV in their blood. Also, Fauci's team could not detect any HIV capable of replicating in resting T-cells cultured from the peripheral blood of six of the 14 subjects who had received IL-2. Even when they cultured a much larger sample of resting T-cells—up to 330 million cells—from each of those six, they still could find no live virus in three of them. In contrast, the team found live HIV in the T-cells from all of the 12 patients receiving combination therapy alone.

Fauci's team went on to perform a lymph node biopsy on one of the three patients who showed no sign of virus in their T-cells. Again, they could find no HIV capable of replication in the lymph node tissue, Fauci says. Although the results raise hopes that eradication of HIV may be a possibility, "we cannot yet conclude we've got eradication of the virus", Fauci says. "The final proof of the feasibility of effectively controlling HIV in latently infected cells will be the discontinuation of combination drug therapy and long-term follow-up", Fauci says, adding that such trials are planned to begin early next year. (Source: *Science*, vol. 282, 20 November 1998)

Innocuous virus turns tumour killer

A harmless and common virus could become an important weapon in the fight against cancer, Canadian biologists claim. Patrick Lee and colleagues from Calgary Health Science Centre in Alberta says that the virus could potentially be used to treat up to 80 per cent of human tumours.

The virus, called a reovirus, produces at worst a mild respiratory and gastrointestinal infection in humans. However, it is naturally primed to infect and kill cells in which the *Ras* signalling pathway, which controls the division and growth of cells, has been activated.

When this pathway goes wrong—if activated by mutated genes, for example—the cell can grow uncontrollably and become cancerous. The *Ras* pathway has been targeted in cancer research before, but only in attempts to block the pathway to stop cancer spreading.

The Canadian team tested reovirus against human and mouse tumour cells implanted into mice. The tumours shrank significantly after being injected with the virus, although repeat doses were needed in mice with healthy immune systems. The virus did not stray into healthy cells, the team reports.

Lee is optimistic about applying reovirus therapy to humans, but admits that it is difficult to predict how many cancers are potentially treatable by the virus.

Ras is activated in around a third of all human cancers, including 90 per cent of pancreatic cancers and 40 per cent of lung cancers. In tests on 25 human cancer cell lines, 20 proved susceptible to reovirus infection.

The team has applied to the Canadian authorities for initial clinical trials on a number of cancers, including breast cancer and head and neck tumours. (Source: *Chemistry & Industry*, 16 November 1998)

Fake viruses as vaccines

Fake viruses made in test tubes have been used for the first time as vaccines, protecting mice against a dangerous brain infection. The Austrian team who developed the bogus viruses predict that they will be as effective as weakened strains of real viruses.

The fake viruses could also be safer than real ones, because in theory they cannot mutate into a fully infectious state. Nor do they have the power to invade cells, making them safer to produce and handle than weakened versions of live viruses.

Christian Mandl and his colleagues at the University of Vienna made the viruses form RNA, the single-stranded cousin of DNA. They genetically engineered *Escherichia coli* bacteria so that they would make RNA molecules with an almost identical structure to the RNA of viruses which cause tick-borne encephalitis, a potentially fatal brain infection. But the fake viruses did not contain some of the genes necessary for the real viruses to cause disease.

Mandl's team then purified the RNA and used it to coat gold beads just one micrometre in diameter. They then fired the beads into cells in the abdomens of mice using a "gene gun". All the mice then survived infection with the tick-borne encephalitis virus.

The researchers say that the immune systems of the mice mistake the fake viral RNA for the messenger RNA which would be made by real viruses when they infect cells.

Mandl and his colleagues expect the RNA vaccines to be superior to DNA vaccines, which have inspired much hope in recent years. DNA vaccines are plasmids, loops of DNA containing copies of viral genes. No one knows exactly how DNA vaccines work, but the genes probably make viral proteins in the body. These trigger immunity, which combats real infections. But there are worries that the DNA could sneak into a person's DNA, perhaps disrupting genes that protect against cancer. This could not happen with RNA.

Mandl says it may be possible to use the same approach for vaccines against yellow fever, dengue fever, polio and hepatitis C. But RNA vaccines might be ineffective against HIV for the same reason that other vaccines fail—because it mutates so fast. (Source: *New Scientist*, 5 December 1998)

Just how do AIDS and Alzheimer's damage the brain?

Poisons released by dying astrocytes—cells that normally nourish nerve cells and detoxify the brain—could solve the mystery of AIDS dementia.

One in five people with AIDS eventually develops dementia. The mystery is that HIV does not appear to infect nerve cells. Until now, the leading theory has been that AIDS dementia is due to the virus infecting microglia, a type of immune cell found only in the brain, which can produce toxic biochemicals such as quinolinic acid.

But a research team from Flinders University in Bedford Park, South Australia, and Johns Hopkins University in Baltimore recently discovered that HIV can also infect astrocytes, although it cannot multiply in them. The researchers wondered if this seemingly passive infection might still contribute to dementia.

They studied four brains from healthy people who had died in accidents, and 18 brains from people who had died from AIDS. The AIDS patients were divided into three groups: those who had never suffered dementia; "rapid dementers" who developed severe dementia over a period of months; and "slow dementers" who took several years to develop severe dementia.

The brains of those with dementia had significantly more astrocytes that had undergone apoptosis, or programmed cell death, compared with those of the controls and AIDS patients without dementia. This trend was especially marked among the rapid dementers.

Once the astrocytes started dying off, the brain would begin to lose its main way of removing glutamate, an amino acid that at high concentrations can kill neurons. The excess glutamate would cause dementia.

Bruce Brew of the National Centre in HIV Research Epidemiology and Clinical Research in Sydney says that the next step will be to show that the death of astrocytes leads directly to the death or damage of neurons. The Flinders-Hopkins team also aims to find out exactly when the astrocytes become infected. (Source: *New Scientist*, 5 December 1998)

Blocking sex hormones might help to restore immunity

Temporary chemical castration could help regenerate the damaged immune systems of people with HIV or who have had chemotherapy or bone marrow transplants. By temporarily blocking sex hormones, suggest researchers in Australia, it may be possible to boost the function of a gland in which vital immune cells develop.

T-cells, which attack virus-infected cells and help coordinate the immune system, develop in the thymus gland, deep in the chest. At puberty, the gland shrinks and its internal structure becomes disorganized, so many researchers thought that it ceased to function.

But Richard Boyd and Jayne Sutherland of Monash Medical School in Melbourne have now shown that the thymus remains active in adult mice. The researches injected a dye into the thymus that marked immature T-cells and traced these cells' migration into the bloodstream. They found that the thymus in adult mice was still releasing T-cells, albeit at about a tenth of the rate it does in a young animal.

Also, when Boyd and Sutherland physically castrated some mice, they found that the thymus regained its youthful appearance within four weeks and that the number of T-cells it produced increased to near pre-pubertal levels.

In a related study, Richard Koup of the University of Texas Southwestern Medical Center in Dallas and his colleagues measured the levels of a genetic by-product of the release of T-cells by the human thymus. Koup's team found that, as in mice, the gland continues to function after puberty at a similarly reduced level.

Koup also has evidence that the increase in T-cell numbers in HIV patients receiving aggressive treatment with combinations of AIDS drugs is caused, at least in part, by the release of T-cells by the thymus. This suggests that boosting the gland's function might help to combat AIDS.

The Australian findings indicate that drugs that suppress the production of sex steroids and partially reverse puberty might boost the immune systems of patients with AIDS or those who have been given immunosuppressive drugs.

With that in mind, Boyd and Anthony Schwarer, head of bone marrow transplants at the Alfred Hospital in Melbourne, are planning to test whether LHRH, or luteinising hormone-releasing hormone, which knocks out the production of sex hormones, also rejuvenates the thymus of adult mice. (Source: *New Scientist*, 19/26 December 1998-2 January 1999)

Ancient virus may be helping HIV buy time to mutate

An ancient virus that invaded the human genome millions of years ago may be an accomplice to HIV, according to a controversial new theory. A biologist says genetic instructions from this "fossil" virus may help HIV evade potent antiviral drugs.

Retroviruses such as HIV insert their own DNA into the genome of living cells they infect. Some experts estimate that as much as 1 per cent of the human genome is composed of fossil retroviruses that infected sperm and eggs millions of years ago. Normally these genes lie dormant, but physicians have noticed that they are sometimes expressed in placental tissue and cancerous tumours.

Some researchers have also wondered if modern-day retroviruses such as HIV could switch on their fossil cousins. And there are hints—albeit indirect ones—that they can. One study has shown that 70 per cent of people infected with HIV have antibodies to a fossil retrovirus called HERV-K, compared with just 3 per cent of uninfected people.

Eric Towler of the Science Applications International Corporation in Maryland, a subcontractor to the National Cancer Institute near Washington, D.C., says he has evidence that HERV-K enzymes may help HIV to evade potent drugs. The idea first came from researchers in Germany who found that HERV-K, like HIV, produces a protease, an enzyme that cuts up long protein chains.

Working together, Towler and the German researchers have shown that in the test tube, the ancient protease cuts a long HIV protein at exactly the same spot as the HIV protease. Towler has also found evidence that HERV-K protease can get into HIV in living cells and gets transformed into an active form of the enzyme.

Towler also tested the effects of various anti-HIV protease inhibitors on HERV-K protease. The fossil protease turned out to be highly resistant to all the drugs. He suspects that when HIV protease is inhibited by drugs, HERV-K's enzyme might take over its work. This could let HIV survive long enough to develop mutations that allow it to resist the drugs' attack.

If cells that are engineered to express HERV-K protease are infected with HIV that makes faulty protease and the HIV survives nonetheless, this would prove that HERV-K is helping the deadly virus along. Towler says his team is carrying out this test at the moment. (Source: *New Scientist*, 19/26 December 1998-2 January 1999)

Even altered HIV could be too dangerous to use as a vaccine

People infected with a weakened form of HIV have finally started to develop signs of AIDS after more than a decade of good health, dashing hopes that a similar mutant virus could be used as a live vaccine.

Vaccine researchers using dead viruses or HIV proteins have largely failed to provoke an immune response that would protect against HIV. But in monkeys, vaccines consisting of a live simian immunodeficiency virus (SIV) in which parts of a key gene called *nef* have been deleted protect against subsequent infections with intact SIV—which is closely related to HIV.

This, together with observations of people infected with forms of HIV with naturally occurring mutations that disable *nef*—who have remained healthy for many years—led some researchers to argue that viruses with deletions in genes such as *nef* are the best hope for an AIDS vaccine.

But further studies of vaccinated monkeys suggest that *nef*-deleted viruses do eventually cause disease. Now that human patients are also becoming ill, the idea may be dead, says David Baltimore of the California Institute of Technology, who heads the AIDS Vaccine Research Committee of the US's National Institutes of Health. "The mutants are not safe enough", he said.

In a paper in the latest issue of *The New England Journal of Medicine* (vol. 340, p. 236), Ronald Desrosiers of the New England Regional Primate Research Center in Southborough, MA, reveals that in 1997 one Massachusetts man infected with *nef*-deleted HIV began to lose large numbers of the CD4 lymphocytes that are destroyed by HIV. He is now taking drugs to treat AIDS.

Similar observations have been made in Australia by researchers at the Macfarlane Burnet Centre for Medical Research in Fairfield, Victoria. But Desrosiers, the main proponent of *nef*-deleted live HIV vaccines, does not accept Baltimore's grim assessment. The strains that might eventually be tested in human volunteers have much more deleted from their genome than just *nef*, he says.

Baltimore hopes it will be possible to design a safe live vaccine. "But it's going to take a lot more subtle development." He fears that deleting more genes will not work. Indeed, new evidence shows that even SIV missing three genes essential for replication—*nef*, *vpr* and *NRE*—can cause AIDS in vaccinated monkeys. (Source: *New Scientist*, 30 January 1999)

Protein therapy may open brave new world in HIV medicine

A new technique—called protein therapy—has the potential to force diseased cells to commit suicide, say researchers.

Protein therapy experiments in the laboratory at St. Louis' Washington University School of Medicine also show greater promise than gene therapy in transducing into cells, the researchers add.

If animal studies continue to show promise, protein therapy in humans could begin within two years, said Steven Dowdy, assistant professor of molecular oncology. He said that until now pharmaceutical treatment of diseases was limited to small molecules which could penetrate the cell membrane.

Dowdy said small molecules have disadvantages in that they could not get into every cell; the smaller molecules often arrested development in normal cells; large numbers of the molecules were required; resistance to the molecules can occur rapidly.

"Using the larger proteins gives us the advantage of exploiting 5 billion years of evolution", Dowdy said, providing far more receptor targets than are available with small molecules.

Dowdy said scientists figured out how to unfold and refold larger proteins to allow these molecules to penetrate cells as early as 1988. But once it was shown to be possible, the work lay fallow until he came up with the idea of using large proteins to attack HIV. HIV, the virus that causes AIDS, uses a scissors-like protein called a protease to cut out enzymes it needs for reproduction.

Drugs called protease inhibitors, which are extending lives of many HIV-infected people, sit in the hinge of the scissors, preventing them from doing their job, but mutations in the protease can make the drugs ineffective. The drugs also inhibit a patient's own proteases, so they are often toxic.

Adita M. Vocero-Akbani, Ph.D., a research associate of the Howard Hughes Medical Institute, and lead author of the study reported in *Nature Medicine*, pieced together a novel protein.

First she took a protein that can slip through cell membranes. Then she attached two pieces of a human enzyme called caspase-3, which, when activated, enables cells to undergo apoptosis.

She joined the three pieces together with cleavage sites from HIV that tell the viral protease where to cut. She then exposed cultured HIV-infected cells to this fusion protein, which was smuggled into all of the cells

by its protein transduction motif—the protein that travels through cell membranes. Because the cells contained actively reproducing HIV, they also contained the viral protease.

This enzyme chopped up the fusion protein at the "Cut here" sites, freeing the two pieces of caspase-3. Capsase-3 activation begins a robust, unstoppable chain reaction of thousands of other capsase molecules in the cell, leading to cell death.

Dowdy said that in the test tube the reaction is so quick that all HIV-infected cells will die within 10 minutes. Because the protein molecule is programmed only to react to HIV-protease, uninfected cells are not affected. And because the reaction occurs so quickly and so far down the line of the cascade, he does not think the molecule will be able to develop a mutation to defend against the protein therapy.

"Even if HIV does somehow come up with a natural defence", Dowdy said the protein can be redesigned to attack that mutation as well.

"This Trojan horse approach should be applicable to many other infectious diseases, such as hepatitis C, malaria and herpes", he said. "We also hope that future modifications will allow us to selectively kill cancer cells."

He cautioned, however, "protein therapy is not a cure for AIDS. We will not even begin human tests for at least a year". Animal studies are already under way. (Source: *Biotechnology Newswatch*, 18 January 1999)

HIV's subtle ways

The idea that HIV makes the immune system "wear itself out" appears to have suffered a fatal blow. The effects of the virus now seem far more subtle.

For years, most people have thought HIV weakens the immune system by destroying CD4 cells, which orchestrate the body's defences. Though the body then speeds up production of new CD4 cells, it fails to replace them all. Dissenters believed the picture is more complicated: HIV causes some CD4 cells to hide away, induces extra programmed cell death, and hits the production of new cells.

To try to resolve the debate, Mike McCune and his colleagues at the University of California, San Francisco, gave injections of radioactively labelled glucose to volunteers with and without HIV. The rates at which the glucose was incorporated into the DNA of dividing CD4 cells were the same for the two groups, implying that production of the cells does not speed up in infected patients.

Giuseppe Pantaleo of Lausanne University in Switzerland says this puts an end to years of debate. It shows treatments for HIV may need to strengthen the immune system, rather than simply target the virus. (Source: *New Scientist*, 9 January 1999)

Chimp version of HIV finally linked to human epidemic

HIV originated in a subspecies of chimpanzee in west Central Africa, an international research team has concluded.

AIDS researchers have long suspected that HIV-1 evolved from one of the simian immunodeficiency viruses (SIVs) that infect other primates. But virologists had never identified an SIV strain similar enough to HIV-1 to be its forerunner.

Now Feng Gao of the University of Alabama in Birmingham and his colleagues have found a match between the three major groups of HIV and SIV sequences from the chimp subspecies *Pan troglodytes troglodytes*. "But these animals don't seem to get AIDS", says Gao, who hopes this may help in designing a vaccine. (Source: *New Scientist*, 6 February 1999)

Research on bacterial genes

Chlamydia genome offers surprises

Analysis of the 1-Mb genome of *Chlamydia trachomatis* has revealed some unexpected biology for the tiny organism. *C. trachomatis* is responsible for causing the most common bacterial sexually transmitted disease (STD) in the United States as well as trachoma, a major cause of blindness in Asia and Africa. A collaboration among scientists at the University of California at Berkeley and Stanford University, the study was reported in the genome issue of *Science* (23 October 1998).

Of 18 fully sequenced bacterial genomes, *Chlamydia* is the only obligate intracellular parasite, growing exclusively within eukaryotic cells and requiring host enzymes and cellular machinery for several necessary functions. Researchers were surprised to learn that it harbours genes that could allow it to generate its own energy-storage molecule, ATP (adenosine triphosphate).

Another new finding explained why *Chlamydia* is vulnerable to penicillin. Although *Chlamydia* was thought to lack peptidoglycan, a vital bacterial cell-wall component and the antibiotic's major target, scientists have identified the genes for synthesizing this molecule. Other genes found for new surface proteins may be important for future vaccine development, possibly by using the gene sequence itself instead of the protein to stimulate an immune response. Data are available on the *Chlamydia* Genome Project Web site (*chlamydia-www.berkeley.edu:4231*).

The project is focusing now on sequencing the genome of the organism *C. pneumoniae*, which causes a mild pneumonia and also may contribute to the development of atherosclerotic lesions. This project is funded by the genome data company Incyte Pharmaceuticals (Palo Alto, CA), which also is sequencing human genes. (Source: *Human Genome News 10(1-2)*, February 1999)

Genome links typhus bug to mitochondrion

As recently as the First and Second World Wars, the louse-borne disease typhus swept through armies, ghettos, and prison camps, killing millions of people. Instability and the breakdown of public health measures in Eastern Europe have experts worrying about possible new epidemics of the disease, which is marked by high fever and delirium. But a close look at *Rickettsia prowazekii*, the bacterium that causes the disease, reveals that, in spite of its fearsome reputation, it is a degenerate organism, riddled with non-functional genes and gradually losing genes it once needed to function.

Molecular microbiologist Charles Kurland of the University of Uppsala in Sweden and his colleagues have sequenced the 1.1-million-base pair genome of the pathogen. By helping identify genes that made *R. prowazekii* so deadly, the information may help researchers design better typhus vaccines. The sequence is also a window to the distant past.

Researchers think that the mitochondria, the small structures that serve as the cell's powerhouses, were derived from bacteria that took up permanent residence in an early ancestor of modern cells. Comparisons of ribosomal RNA genes had indicated that *Rickettsia*, one of the so-called alpha proteobacteria, could be the closest living relative of the mitochondria's predecessor. Now, Kurland says, the genome sequence "is as confirmatory as you can imagine" about the link between mitochondria and *Rickettsia*. It also illustrates the gene loss that must have marked the mitochondrion's own transition to dependence on the host cell.

Kurland and his colleagues, who began the sequencing project six years ago, found 834 genes in the *Rickettsia* genome, a half-dozen of which code for proteins similar to those that make other bacteria virulent. Three of these look like the genes that produce toxic polysaccharides in *Staphylococcus aureus*, which causes boils. The information should help researchers interested in developing new vaccines for typhus find the right proteins to include in their inoculations, Kurland says.

The effort also seems to have paid off in helping pin down the origins of the mitochondria. With the sequence in hand, Kurland, Uppsala's Siv Andersson, and their colleagues compared the *Rickettsia* genes to the DNA still present in modern mitochondria. "We see very strong similarities", says Andersson, particularly in genes involved in energy production. The group also found that many of the pathogen's genes closely resemble genes that code for proteins used by yeast mitochondria—but are found in the nucleus of yeast cells.

This suggests, Kurland says, that somehow "there was an early evolutionary event where there was an off-loading of these genes" from the early mitochondrion to the nucleus. As the ancestral host nucleus took on these genes, the mitochondria would have become more dependent on the host cell, until eventually they could no longer survive except within the cell. (Source: *Science*, vol. 282, 13 November 1998)

Training a molecular gun on E. coli

Researchers at the National Institutes of Health (NIH) are closing in on the development of the first vaccine against *Escherichia coli* O157:H7, a pathogenic version of the common gut bacterium. Tests of an experimental vaccine showed promise in adults, and the researchers are about to apply for approval to test it in young children. If the trial gets the go-ahead and the preparation passes further tests, experts say, a vaccine for people and one for livestock could be available early next century.

First identified in 1982, O157:H7 made headlines five years ago when contaminated hamburger meat sickened more than 500 people, triggering symptoms such as bloody diarrhoea and kidney failure. Since then, the bacterium has turned up sporadically in everything from raw milk and apple juice to daikon radishes and drinking water. Some 20,000 cases occur each year in the United States, resulting in about 250 deaths; young children are the main victims. Moreover, O157:H7 shrugs off antibiotics with ease.

To tackle this daunting public health threat, a team led by NIH immunologist Shousun Szu is combining cutting-edge molecular biology with a method that dates back to Louis Pasteur. They homed in on O-specific polysaccharide, a molecule that studs the bacterium's cell membrane "like hair on the scalp", Szu says. Its structure is unique to O157:H7, she says, and thus serves as a good vaccine target.

The team tested the conjugate vaccine in adults. Within four weeks, all 87 volunteers had substantial blood levels of antibodies to the O157:H7 polysaccharide, with no observed side effects. More importantly, the subjects' blood serum contained enough antibodies to kill O157:H7 bacteria, even after being diluted at least 1000-fold. For the next step, Szu's group is preparing to submit to an NIH safety panel a protocol for a similar study in 60 children aged two to four.

While clinical trials press ahead, Szu's team is hoping to design and test an O157:H7 vaccine in cattle—up to two per cent carry the bacterium in the United States. In cattle, however, O157:H7 does not attach to the gut lining like it does in people, where it is easily reached by antibodies. It is unclear whether cow antibodies can reach the free-swimming bacteria in the intestines and stomach. (Source: *Science*, vol. 282, 20 November 1998)

Bacteria in termites curb methane output

Corkscrew-shaped bacteria that live in termite intestines curb the insects' output of methane, microbiologists have found. The discovery hints at possible ways of preventing other microbial ecosystems from releasing methane, which is an important greenhouse gas.

Termites' guts teem with hundreds of species of protozoans, bacteria and archaeans. Among the most abundant of these single-celled inhabitants are the spiral bacteria known as spirochaetes. But for decades, no one knew what they did, because efforts to grow them in laboratory cultures always failed.

Jared Leadbetter, a microbiologist at Michigan State University in East Lansing, hit on the right combination of nutrients and antibiotics in which two spirochaete species grew but other bacteria did not.

The newly cultured bacteria turn out to have a key position in the intricate microbial food chain within the termite gut. Various microbes, together with enzymes secreted by the termites themselves, break down the cellulose and other large molecules in wood fibres into acetate—the insects' main energy source—plus hydrogen gas and carbon dioxide. Studies of the spirochaete cultures show that the bacteria convert these gases into more acetate. This process yields about a third of the acetate available to the termites.

The spirochaetes may have an even further reaching effect, because they prevent other microbes from using the hydrogen and carbon dioxide to make methane, which is an important contributor to global warming. This finding could lead to ways of reducing methane production in other important microbial ecosystems, such as those in the guts of cattle and other ruminant grazers. (Source: *New Scientist*, 6 February 1999)

A newly discovered bacterium could digest a fuel additive polluting groundwater

Attempts to solve one of the most serious groundwater pollution problems in the US have been boosted by the discovery of a microorganism that can degrade methyl *tertiary*-butyl ether (MTBE), an evil-smelling, possibly carcinogenic fuel additive.

MTBE is added to petrol in the US to improve combustion and reduce air pollution. It is credited with halving emissions of the carcinogen benzene from exhausts and its use has improved air quality in some of the country's smoggiest cities.

But the chemical has leaked into aquifers from thousands of underground petrol storage tanks. It is highly water-soluble and long-lived, and is classified by the Environmental Protection Agency as a potential carcinogen. So disagreeable is its smell that even minuscule amounts can render water virtually undrinkable.

Faced with contaminated drinking water supplies and increasing public pressure to ban the additive, the state of California—the worst affected—appealed to its universities for a solution. Ed Schroeder and Juana Eweis at the University of California at Davis noticed that something in a compost tray at a water treatment plant near Los Angeles was eating airborne MTBE. Soil microbiologist Kate Scow and graduate student Jessica Hanson isolated the bacterium responsible. The species has not yet been identified and is currently known as PM1.

In laboratory studies, Scow injected the microorganism into samples of contaminated soil. She found that at a concentration of a million cells per gram of soil, the bacteria digest a dose of MTBE of 25 parts per million in about six days.

Engineers have developed bioreactors for cleaning up contaminated water, but cleaning up groundwater *in situ* may prove more difficult. For instance, the researchers are still not sure how the bacteria will perform in the natural environment when other chemicals are present. (Source: *New Scientist*, 19/26 December 1998-2 January 1999)

Food poisoning bug Salmonella tamed, trained to fight cancer

Gene engineers have tamed *Salmonella typhimurium*, turning it into a potent cancer inhibitor that does not harm non-cancerous cells. Mutant Salmonella stopped tumours from growing and doubled the survival time of experimental mice in a new study. Trials of the bacterial cancer therapy in humans could start in the first quarter of 1999, said Dr. David Bermudes.

The approach will most likely be used first on melanoma, but Bermudes said the gene-altered germs also attack other major killers, like breast, colon and lung cancer.

Bermudes is the associate director of biology at Vion Pharmaceuticals, Inc., the New Haven, CT-based biotechnology company that is developing the technology with collaborators from nearby Yale University School of Medicine, Texas A&M University and the University of Washington.

Bermudes says the approach makes use of Salmonella's natural preference for cancer cells. Without any gene alterations, he said, Salmonella will infect tumours at levels about 100 times higher than healthy tissues.

He says scientists discovered that bacteria had the potential to combat cancer about two centuries ago, when they observed that tumours would sometimes shrink in patients who had serious infections.

They could not make use of it because the germs attack healthy cells as well as tumours, making the therapy lethal. But with the tools of molecular biology, scientists can cut out the lethal traits while holding onto the cancer fighting properties.

In mice, the therapeutic dose is at least one hundred times smaller than the toxic dose.

"We see little, if any, side effects", said Bermudes.

Starting in about 1993, Bermudes and his research team began tinkering with the bacteria, first removing its ability to make an essential building block for DNA called purine. Purine dependent Salmonella had an even greater hunger for cancer cells, concentrating in tumours at levels that were a thousand times higher than in normal tissues.

This made it possible to consider the bacteria as a therapy, because anti-cancer effects could be seen with injections of germ, but in amounts so small that they could not cause dangerous infection, he said.

But before it could be tried on humans, the scientists had to engineer in a second safety mechanism, to avoid another life threatening complication—septic shock.

In the new study, the scientists cut out part of a gene called msbB. This gene produces "lipid A", a molecule composed of fats and sugars on the surface of the germ's cells. They could only trim the gene, because Salmonella cannot survive without it.

The complete lipid A provokes production of tumour necrosis factor-alpha (TNF-alpha). When the body produces too much TNF-alpha, it can lead to septic shock and ultimately cause organ failure and death.

By altering lipid A, the scientist created a form of Salmonella that will attack cancer, but not hurt the patient.

In the current study, the scientists tested the Salmonella as a cancer treatment in mice, but also injected the gene-altered bacteria into pigs to gauge its safety.

Bermudes said the immune systems of pigs and humans have similar strong reactions to lipid A, which mice lack. In the research, the genetically modified microbes did not produce septic shock in pigs, he says, which bodes well for the therapy in humans.

Bermudes said he is not sure why the therapy works. It is possible that the bacteria promote apoptosis, or programmed cell death, or that they starve cells by competing for nutrients.

One advantage to the therapy is that it will work in both primary and metastatic tumours, because Salmonella can seek out the tumour cells. Other microbial therapies have to be injected directly into the tumours.

The new technique, however, is potentially very important, because it offers a new approach to treating a disease that kills more than half a million people a year. (Source: *McGraw Hill's Biotechnology Newswatch*, 4 January 1999)

Striking similarities seen in ulcer bug strains

The first comparison ever of the genomes of two strains of the same bacterium—*H. pylori*—shows a surprising similarity between the two, with a relatively small amount of variation. The study was a comparison of two sequences of *H. pylori*, one from The Institute for Genome Research (TIGR), Rockville, MD, and another from that of Genome Therapeutics Corp. of Waltham, MA.

The TIGR sequence came from a patient with a duodenal ulcer, and GTC's was from a gastritis patient. The comparison showed that only 6 to 7 per cent of the genes are specific to each strain, according to the study published in *Nature*.

This research represents a major application of genomics to drug discovery, with the study yielding information that surprised scientists. "Contrary to what has been widely believed, the genomic variance in *H. pylori* is relatively limited, and a disproportionate fraction of the strain-specific genetic variances are physically clustered", said Richard Alm, Ph.D., lead author on the paper and research scientist at Astra Research Center in Boston, Genome Therapeutic's corporate partner for *H. pylori*.

"By comparing the genomic sequences for two strains of this pathogen, we have created a new framework for more fully understanding the biology of *H. pylori*. This framework is enhancing our ability to analyse and interpret the factors that affect infection and the disease process", he added.

The research, conducted by scientists at Genome Therapeutics and Astra Research Center in Boston, is directed toward discovery of new targets to treat *H. pylori* infections. The two companies are working together under an agreement to develop vaccines and small molecule drugs to treat *H. pylori* infection, which affects 50 per cent of the world's population and is considered to be the cause of gastritis, peptic ulcers and stomach cancers. Current antibiotic treatments for *H. pylori* are less than effective, with the number of strains resistant to the pathogen growing. (Source: *McGraw Hill's Biotechnology Newswatch*, 1 February 1999)

Does a bacterium turn the body against itself?

Cardiac problems linked to infection with *Chlamydia pneumoniae* may be a symptom of an autoimmune disease. An international team believes the immune system is fooled into attacking the heart because the bacterium carries a sequence of four amino acids that are also found in an important heart protein.

Several studies have linked *C. pneumoniae* infections to heart disease, but no one has explained how the bacterium could damage the heart, especially since it infects the lungs or reproductive organs.

Now a multinational team led by Josef Penninger of the Ontario Cancer Institute in Toronto believes that *Chlamydia* can trigger an autoimmune disorder. The researchers were originally investigating a Coxsackie virus that causes a rare kind of heart failure in young people. The virus appeared to trigger an autoimmune response to a heart muscle protein called myosin. So the researchers spent two years screening peptides in the myosin protein to see which of them caused autoimmune disease when injected into mice. They eventually found a sequence of four amino acids that was especially potent.

Trawling a database of viral and bacterial peptide sequences for the same motif, the team unexpectedly found that the sequence matched peptides on the cell surface of three different species of *Chlamydia*, rather than peptides produced by the Coxsackie virus. When the team took these peptides and injected them into mice, the animals all developed inflammatory heart disease.

Penninger believes that his group's discovery will lead to better treatments for heart problems caused by *Chlamydia*.

At least two large clinical trials are currently giving antibiotics to thousands of people to see if this decreases their risk of developing heart disease. If Penninger is right, antibiotics will not work unless they are given very early in an infection, before the autoimmune response kicks in. He believes the best solution may be a *Chlamydia* vaccine that leaves out the sequence of four amino acids, so that the immune system can be trained to

fight the bacterium without producing antibodies that also attack the heart. (Source: *New Scientist*, 6 March 1999)

Unique activity associated with non-insecticidal Bacillus thuringiensis parasporal inclusions: in vitro cell-killing action on human cancer cells

The Biotechnology & Food Research Institute, Fukuoka Industrial Technology Center, Kurume, and Institute of Biological Control, Kyushu University, have discovered that a unique cytocidal activity against human cancer cells is associated with non-insecticidal parasporal inclusions of certain *B. thuringiensis* strains.

Parasporal inclusion protein from a total of 1744 *Bacillus thuringiensis* strains, consisting of 1700 Japanese isolates and 44 reference type strains of existing H-serovars, were screened for cytocidal activity against human leukaemia T cells and hemolytic activity against sheep erythrocytes. Of 1684 *B. thuringiensis* strains having no hemolytic activity, 42 strains exhibited *in vitro* cytotoxicity against leukaemia T cells. These non-hemolytic but leukaemia cell-toxic strains belonged to several H-serovars including *dakota*, *neoleonesis*, *shandogiensis*, *coreanesis* and other unidentified serogroups. Purified parasporal inclusions of the three selected strains, designated 84-HS-1-11, 89-T-26-17, 90-F-45-14, exhibited no hemolytic and no insecticidal activity against dipteran and lepidopteran insects, but were highly cytocidal against leukaemia T cells and other human cancer cells, showing different toxicity spectra and varied activity levels. Furthermore, the proteins from 84-HS-1-11 and 89-T-26-17 were able to discriminate between leukaemia and normal T cells, specifically killing the former cells. This finding explores the untouched world of this organism, and may lead to the use of *B. thuringiensis* inclusion proteins for medical purposes.

Bacillus thuringiensis was first isolated in Japan as a pathogen of the sotto disease of the silkworm, *Bombyx mori*, early in this century. The organism is a Gram-positive, spore-forming bacterium that produces crystalline parasporal inclusions during sporulation. The inclusions often exhibit strong insecticidal activity against several orders of insects, making *B. thuringiensis* a reliable agent for microbial control of insect pests of agricultural and medical importance. The insecticidal parasporal inclusions contain two families of insect-toxic molecules, Cry and Cyt proteins. The Cry protein is specifically toxic to insects and is currently classified into twenty genetically different major groups, Cryl to Cry22. The high specificity of Cry proteins in killing insects is attributable to specific binding of the proteins to receptors that reside on the midgut cell membranes of susceptible insects. Another family of the toxin, the Cyt protein, has a broad cytolytic activity against invertebrate and vertebrate cells including erythtocytes, and is divided into two genetically different groups, Cytl and Cyt2 (formerly CytA and CytB, respectively).

Further details from: Biotechnology & Food Research Institute, Fukuoka Industrial Technology Center, 1465-5 Aikawa-machi, Kurume, Fukuoka 839-0861. Tel.: +81-942-30-6644. Fax: +81-942-30-7244. (Source: *JETRO*, February 1999)

Saviour from the acid swamps

A bacterium that digests methane is doing its bit to slow global warming, say the American, German and Russian researchers who discovered it, but they warn that the bacterium—the first of its kind to be found in acidic wetlands—is being poisoned by industrial pollutants.

"The bacterium is a real novelty in two ways", says Werner Liesack, a team member form the Max Planck Institute for Terrestrial Microbiology in Marburg. It is unrelated to other methane-eating bacteria, and it thrives in acidic conditions.

Although most of the bacteria that produce methane live in acidic wetlands in the northern hemisphere, these environments were thought to be unsuitable for bacteria that digest the gas, but the scientists noticed that some wetlands in Europe were only producing about half as much methane as expected—and this led them to the bacterium. Almost half the world's methane emissions come from wetlands in the northern hemisphere.

The bacterium is under threat, however. Nikolai Panikov and Svetlana Dedysh of Moscow University found that it is especially sensitive to nitrate and sulphate pollution from industry and traffic. They say that the methane output of acidic wetlands is now higher than it was before the industrial revolution, because of the decline in this methane-eating bacterium. The team is still deciding what to call the bacterium. (Source: *New Scientist*, 20 March 1999)

Research instrumentation

Gene chip for toxic tests

Drug and chemical group Zeneca is poised to launch a set of revolutionary DNA chips designed to simplify agrochemical toxicology testing to the level of reading a barcode.

The company believes that the chips could help raise its "hit rate" with experimental agrochemicals to more than 80 per cent by weeding out dangerous compounds very early in the development process.

Each chip carries a set of human genes involved in a specific toxicological pathway, such as allergy. Exposing the chip to a new pesticide generates a genetic response, with some genes becoming more active and others switching down. These reactions can be measured to produce a distinctive profile of gene expression.

Zeneca hopes to be using the "toxicogenomic" chips routinely within a year. Company scientists are currently testing prototypes and selecting the sets of genes that are relevant to agrochemical screening.

Although the technology will not replace full-scale testing, Zeneca believes it will eliminate fruitless research projects at an early stage.

The chips have also been earmarked for screening experimental drugs. (Source: *Chemistry & Industry*, 15 March 1999)

Maths to solve human genes

GeneMark, claimed to be the world's most-used software for deciphering the DNA in bacteria, has recently been enhanced to analyse DNA in higher organisms.

GeneMark was developed by Mark Borodovsky, a Russian scientist who now works at the Georgia Institute of Technology, USA. His software uses probabilistic mathematical models to predict the location of genes on a strand of DNA. The latest version, which is called GeneMark.hmm, includes an additional mathematical modelling technique known as Hidden Markov Models (HMM).

The software helps biologists to determine the genetic significance of strands of DNA once they have been sequenced. Understanding the genomes of key microorganisms may give an increased knowledge of human genetics as some of the genes are very similar.

Human DNA is more complicated to understand than bacterial DNA because it is much longer. It also contains "spacers" of non-genetic material between the genes. The locations of these spacers are more difficult to detect using computer algorithms. The latest version of GeneMark has been written to address this problem. (Source: *Scientific Computing World*, February/March 1999)

Saved by the light

Sunlight might one day offer developing countries a cheap alternative to expensive medical lasers, say researchers in Israel. By collecting sunlight in a parabolic dish and feeding the light energy down an optical fibre into an operating theatre, they say it should be possible to carry out many of the techniques normally done by laser—as long as the Sun keeps shining.

Physicists Jeffrey Gordon and Daniel Feuermann of Ben-Gurion University in Beersheva say that many medical laser treatments do not require the power of lasers. If tissue does not have to be cut, they say, all that is needed is for the tissue to absorb high levels of radiation. So the pair propose that intense sunlight could be harnessed to provide energy for therapies as diverse as treating skin tumours and diseases, tissue welding and angioplasty, in which powerful light destroys plaque in arteries.

The lasers used in medical applications typically deliver light flux densities of up to 100 watts per square millimetre, whereas the solar surgery unit proposed by Gordon and Feuermann could deliver between 30 and 70 watts per square millimetre. They say that this is enough to destroy some tumours: "The key issue in most conventional treatments in killing malignant cells is simply heating it up to approximately 60°C—and to do it quickly and with great precision", says Gordon.

The heart of their system is a parabolic mirror 20 centimetres in diameter. A commercially available suntracker, such as those used for solar panels, keeps it facing the Sun.

Just below the focal point of the dish, a flat mirror reflects the concentrated light onto the tip of an optical fibre. The fibre, which can be up to 100 metres long, links the dish to the operating room.

If surgeons need more power, the light can be concentrated further. This can be done either at the roof-top end, by adding a funnel-shaped extension to the tip of the fibre to gather more light rays from the flat mirror, or in the operating room. Here, the light intensity is increased using a specially shaped optical-fibre tip that ensures that all rays converge on a small area and are not scattered back into the fibre.

The Ben-Gurion team believe their system can be put together for a few thousand dollars, compared with more than $120,000 for a laser. Gordon says that the system would be used mainly in developing nations and field hospitals. He calculates that solar surgery would be feasible "in clear climates" on half the days of the year, for about 7 to 10 hours a day. (Source: *New Scientist*, 27 February 1999)

Turing in the genes

Nippon Electric Corporation researchers in Princeton, NJ, have patented (US 5 804 373) a universal computer, originally proposed by British codebreaker Alan Turing. This one stores programs on DNA strands instead of an infinite tape. NEC uses circular loops of DNA, where a sequence of 20 molecules represents a letter of the alphabet. The loops are cut with enzymes to create "sticky" ends which can be re-joined to explore every possible combination of letters. Staining the DNA with dye will reveal the patterns formed. Although DNA computing is slow, a very large number of strands can be treated at the same time to create a massively parallel processor. (Source: *New Scientist*, 28 November 1998)

DNA circuits

DNA could soon be used to link components in tiny circuits if Nanotronics of San Diego and the University of California are successful with their joint patent application (WO 98/28320). Very small devices are coated with a predetermined DNA sequence, and the silicon chip acting as the substrate is coated with complementary DNA sequences. When the devices and the substrate are brought together in solution, the complementary DNA sequences bond to each other. The system can be used to deposit a nanomatrix of, for instance, light-emitting devices over a large surface to make a display panel. The finished devices can be either flat or three-dimensional. (Source: *New Scientist*, 14 November 1998)

Vibrating cells could be the ultimate in non-invasive screening

The quest for a universal signature for cancerous tissue may have taken a step forward with a fast three-dimensional imaging device. This uses ultrasonic emissions to highlight variations in electrical conductivity between healthy and diseased tissue, and it could also avoid the need for painful biopsies.

The technique, called Hall Effect Imaging (HEI), relies on the interaction between ultrasonic vibrations and strong magnetic fields to map the dielectric properties of the body. The technique's inventor, Han Wen, who is based at the National Institutes of Health in Bethesda, MD, hopes his HEI will complement magnetic resonance imaging (MRI) which uses nuclear magnetic resonance to produce tissue density maps of the body.

"Some published results suggest that breast tumours trigger large changes in tissue electrical parameters", he explains. This technique should work with other cancers since this change happens in all other tumours, he adds.

HEI works when an oscillating electric pulse is sent through the body while it is exposed to a strong magnetic field. This makes charged particles in the tissue vibrate. If the frequency of the pulse is high enough, then the vibrations can be detected using ultrasound sensors. By monitoring the intensity and the phase difference between the two signals a high contrast 3D image of the tissue inside the body can be constructed in real time.

Wen discovered the effect accidentally when he noticed unexpected electrical activity during MRI scans.

"The technique of choice for screening breast cancer is X-ray mammography", says Aaron Fenster, director of imaging in the Robarts Research Institute at the University of Western Ontario, London, Canada. He reckons that it will be some years before mammography will be replaced because it has such a high sensitivity, but he says this doesn't rule out the use of HEI.

HEI, says Fenster, appears to have one major advantage over existing techniques. HEI has a high specificity which means that false positivities can be ruled out. At the moment, the only way to be completely sure is to take a biopsy.

This is precisely what Wen hopes to avoid with his invention, but HEI is still very much in its infancy. So far, Wen has only successfully tested tissue in the lab: he says it remains to be seen whether the same conductivity differences can be detected in the body. Wen has put together hand-held scanners in his laboratory but needs to build a full-body scanner.

A British company, Oxford Instruments, has started building the super-magnet needed for the job. Although it is relatively small with a diameter of about 1.5 metres, it will have a massive magnetic field of 6 tesla and will take about a year to build. (Source: *New Scientist*, 16 January 1999)

Studying life's little reactions

Chemists at Stanford University, working with researchers at the University of Goteborg and Pomona College in Claremont, CA, US, have found a way to make cell-sized containers in order to study the chemical reaction of biological molecules in an environment that closely mimics that of real life.

The scientists have created tiny membrane sacs, called vesicles, out of organic phospholipid bilayers that closely resemble the membrane structure of specific living cells. Each vesicle contains a single chemical compound.

The minute size of the vesicles (50-2000nm) and limited quantity of their contents (10^{-18}-10^{-21} mol) enables sample manipulation and detection on a scale beyond the scope of traditional analytical methods. Chemical reactions within these vesicles are said to closely resemble that within living cells.

Miniscule chemical reactions can be produced in two ways.

The vesicles can be immersed in a liquid containing a second chemical that will react with the chemical that the sacs contain. Or the vesicle can be positioned between two electrodes using laser-based optical tweezers. The membrane sac would then be zapped with a mild electrical pulse that causes pores to open in the membrane wall and chemicals on either side of the membrane barrier to mix.

Many reactions that take place within a cell do not work in the same way at larger volumes. This is because the molecules inside the cell, driven by thermal energy, are continuously careering off each other and bouncing off the cell's membrane wall.

Using different fluorescent dyes, the researchers estimate that a single enzyme and a single substrate in a moderately sized vesicle will bounce off each other about 300,000 times per second. The substrate will bounce off the membrane about 200 million times per second.

Possible applications of these cell-like containers include investigating aspects of cell metabolism, examining the biochemistry of cells infected with pathogens, and delivering drugs and genes to single cells. (Source: *European Chemical News*, 29 March-4 April 1999)

General

Gut reaction

Fears that genes for antibiotic resistance could jump from genetically modified foods to bacteria in the gut may be fuelled by new research from the Netherlands. The results show that DNA lingers in the intestine, and confirm that genetically modified bacteria can transfer their antibiotic-resistance genes to bacteria in the gut.

Using an "artificial gut", the Dutch researchers showed that DNA remains intact for several minutes in the large intestine.

One concern about some genetically modified (GM) crops, such as maize used as animal fodder, is that they include a gene for antibiotic resistance. The resistance genes are used to track the uptake of modified genes, and are not expressed in the crops. While some scientists fear

that these genes could jump into bacteria in the guts of livestock and create antibiotic-resistant pathogens, others have said there is no such risk because the modified DNA breaks down quickly. The results by Hub Noteborn of the State Institute for Quality Control of Agricultural Products in Wageningen, who helped organize the research, cast doubt on these assurances.

The computer-controlled artificial gut, dubbed TIM, was designed by Robert Havenaar and his colleagues at the TNO Nutrition and Food Research Institute in Zeist to mimic the digestion of food. It provides a mechanical model of the stomach and intestines, and contains the normal microbes and enzymes in the gut.

When TIM was used to study the effects of digestion on bacteria engineered to contain antibiotic-resistance genes, Havenaar found that DNA from the bacteria had a half-life of 6 minutes in the large intestine. "This makes it available to transform cells", he says.

If the modified bacteria were a type normally found in the gut, such as *Enterococcus*, the experiment showed each had a 1 in 10 million chance of passing DNA containing antibiotic-resistance genes to an indigenous gut bacterium when they came in contact. There are normally around a thousand billion gut bacteria, suggesting many would be transformed. If some normal gut inhabitants were killed off—as in the guts of people or animals on antibiotics—the transfer rate from gut-type bacteria increased tenfold.

Bacteria nor normally in the gut, such as *Lactobacillus*, did not transfer antibiotic-resistance genes to a normal population of gut bacteria at a detectable level, according to a TNO internal report. Nor did the Flavr Savr tomato, engineered by the California-based company Calgene to resist rot, although up to 10 per cent of its DNA reached the colon. The researchers hope to carry out the crucial test of whether foreign bacteria and GM foods transfer their genes when gut microbes are depleted. (Source: *New Scientist*, 30 January 1999)

Scientists hunt SNPs to uncover variation, disease

Why does one man live to celebrate his hundredth birthday with a glass of wine in one hand and a cigar in the other while another succumbs in midlife to cancer or heart disease? And why may one woman's breast cancer be effectively eradicated while another's shows no significant response to the same treatment?

The explanations may reside in the cumulative effect of a small number of differences in DNA base sequence called single-nucleotide polymorphisms (SNPs), which underlie individual responses to environment, disease, and medical treatments.

SNPs are the most common type of sequence variation. Other variations include the number of base insertions and deletions and sequence repeats (called mini- and microsatellites). Some disease-causing mutations are SNPs, for example, the single base change in the gene associated with sickle cell anaemia. SNPs occur inside and outside genes, about once every 100 to 300 bases throughout the human genome.

DNA variations are important in understanding the genetic basis for disease and individual responses to environmental factors, as well as for such normal variations in biological processes as development and ageing. For this reason, scientists in the public and private sectors are beginning to focus their attention on methodically searching for SNPs throughout the human genome.

In 1997 the NIH National Cancer Institute launched a Genetic Annotation Initiative to gather SNPs in regions of thousands of cancer-associated genes (*www.ncbi.nlm.nih.gov/ncicgap*). In another NIH programme, a 1998 RFA involves 18 institutes interested in developing genomic-scale technologies or in implementing projects to catalogue and detect SNPs in different DNA samples (*www.nhgri.nih.gov/Grant_info/Funding/RFA/rfa-hg-98-001.html*).

SNPs generated in these public projects will be freely available from dbSNP, a new database at the NIH National Center for Biotechnology Information, which serves as a central repository for SNPs and for short-deletion and insertion polymorphisms (*www.ncbi.nlm.nih.gov/SNP*). (Source: *Human Genome News 10(-2)*, February 1999)

Researchers reengineer penicillin

A research team from the University of Limerick has found a way to reengineer penicillin. The researchers discovered a way to attach an extra molecule to penicillin which is easily sliced off by a bacterial enzyme. This molecule can be designed to become lethally toxic to the bacterium as soon as it is detached. The new form of penicillin can kill both resistant and non-resistant bacteria.

Dr. Timothy Smyth who heads the team said the work was "the first steps toward realizing a new approach to combat bacterial resistance". However, he cautioned that a lot more research was needed before the technique could be used to create a safe and useful drug. (Source: *Irish Biotech News*, December 1998)

Two proteins make list of natural anti-angiogenesis compounds

California researchers have isolated two new proteins that appear to be potent inhibitors of angiogenesis, the all important process of growing new blood vessels.

A team from the University of California in Los Angeles (UCLA) found the new proteins, which they call Meth-1 and Meth-2, by first looking at another protein, called Thrombospondin-1, that has already been proven to inhibit angiogenesis.

They then screened for proteins with similar structures, said Luisa Iruela-Arispe of UCLA's department of molecular, cell and developmental biology.

Preclinical experiments have shown that the proteins they discovered appear to be 20 times more powerful in limiting blood vessel growth than TSP1, she says.

Her team is currently comparing one of the proteins, Meth-1, to endostatin, one of the two natural angiogenesis fighters that has had extremely promising results in animal studies.

Dr. Michael O'Reilly, who discovered both drugs while working with Judah Folkman at Boston's Children's Hospital said that the clinical trials to show whether these drugs will work in humans at all are likely to start in the second half of 1999.

O'Reilly said it took "200 litres of mouse urine" to first isolate and purify angiostatin. Endostatin was found in culture, avoiding the need for an ocean of mouse urine, but it has proven to be unstable and difficult to produce.

At the same meeting, O'Reilly said that new experiments in his laboratory suggest that these proteins work by pushing the tumour into a state of dormancy. They remain in the body, but do not grow. This may explain why some patients who appear to be cured of cancer have a recurrence years, even decades, later.

Natural anti-angiogenesis factors may explain this mystery. Autopsy studies have shown that tumours are present in people who do not appear to have cancer, suggesting that people may have dormant tumours that never cause illness. This raises the possibility that current approaches to fighting the disease may be flawed.

In his laboratory, O'Reilly said that he has experimented with repeated courses of therapy with angiostatin and endostatin to see if the drugs will cause resistance and if the treatments will have to continue for a lifetime to prevent cancer from coming back.

In some cancers, after two to six cycles of endostatin, he found that "dormancy is self-sustained and tumours remain in the body, but as a small, dormant nodule".

In another, yet unpublished part of the research, O'Reilly treated the apparently dormant tumours with growth factors to kick-start them.

The treatment got them growing again, said O'Reilly, proving that the nodules were really left-over cancers.

This research suggests that the treatment may start with an early bombardment with the drugs to stun the tumour into dormancy. Then the therapy may stop. Later, if the tumours start to grow again, doctors may simply be able to repeat the process. It might take years for the cancer to naturally start up again.

O'Reilly said the newly identified proteins are probably part of a repertoire—a "small family"—of natural compounds that keep tumours in check.

That means, like most cancer therapies, anti-angiogenesis drugs will most likely be used in a cocktail of several drugs. The cocktail may have to be tailored to different people and tumour types. (Source: *McGraw Hill's Biotechnology Newswatch*, 16 November 1998)

Organs take sides

A single gene may choreograph the positions of our organs by directing cell division, say researchers in Germany.

In vertebrates, organs inside the chest and abdomen are arranged asymmetrically. The apex of the heart points towards the left, for instance, while the large intestine curls from right to left. Biologists have found several signal molecules that seem to determine the right and left sides at very early embryonic stages. But how they do this is not clear.

Martin Blum of the Karlsruhe Research Centre and his colleagues have discovered a clue to what happens. They found that a gene called *Pitx2* was switched on in cells destined to become the left sides of the heart and gut in developing embryos of mice, frogs and zebra fish. The gene stayed switched on as these organs developed.

The team also tested the effects of *Pitx2* on the right-hand sides of frog embryos by injecting messenger RNA corresponding to the gene. Some of these embryos developed mirror images of normal organ arrangements, while other developed extra-large hearts and guts.

"Basically, *Pitx2*, could serve as a mediator between the signal molecules and the forming organs", says Blum. The signal molecules may switch on *Pitx2* before disappearing, leaving the gene to carry out their instructions as organs develop.

Blum thinks the gene works by controlling cell proliferation. If more cells grow on one side of the gut than the other, for example, the developing organ will begin to curl. (Source: *New Scientist*, 27 February 1999)

Copying DNA is no moveable feast

Genetic material is churned out in DNA factories anchored to cell membranes rather than by roving enzymes as previously supposed.

Before a cell divides, it replicates all its DNA so each daughter cell will inherit a copy of its genes. DNA polymerase, the complex of enzymes that copies DNA, was thought to travel along the double helix like a train on tracks.

But it was also possible that DNA polymerases stayed in one spot like a fixed DNA factory.

To find out, Alan Grossman and Katherine Lemon, of the Massachusetts Institute of Technology, attached a green fluorescent protein from jellyfish to DNA polymerase in the bacterium *Bacillus subtilis*. As the bacteria divided, they tracked the position of the polymerase complexes within the cell by their green glow.

The polymerases replicated the bacterium's genes without straying from their spot next to the cell membrane (*Science*, vol. 282, p. 1516). Grossman believes fixed DNA factories are probably at work in human cells too. (Source: *New Scientist*, 28 November 1998)

Supergenes

Maxygen, a company in Santa Clara, CA, has used a DNA shuffling technique to create interferons that are dramatically more effective against viruses than any produced naturally by the immune system. It has also made ultra-efficient versions of an industrial enzyme.

When organisms reproduce sexually, the offspring end up with a mix-and-match set of genes inherited from both parents. Maxygen's technique is similar, except the

parents are a series of related genes. These are cut into pieces, shuffled together and then assembled to form a new genetic generation. Some of these daughter genes can manufacture proteins that are much better at certain tasks than nature's originals. The best ones can be screened out and shuffled to produce whole lineages of superior descendants, in a process mimicking evolution by natural selection.

The potential of Maxygen's technique is beginning to be realized. The parent genes are first broken into fragments by shattering their DNA with ultrasound, or cutting them up with an enzyme called DNAse. They are reassembled into daughter genes, comprising fragments from several parents, using a variant of the DNA-building polymerase chain reaction. Short template or "primer" sequences ensure that the fragments are stitched together in the correct order to produce a functioning gene. The daughter genes can then be inserted into bacteria or fungi, where they begin making protein.

To make superior versions of an industrial enzyme, Maxygen's scientists isolated genes from 26 microorganisms which each make their own versions of the enzyme. Using its system of DNA shuffling, Maxygen made 600 new daughter genes, 77 of which produced superior enzymes. Screening showed up variants which functioned better than natural enzymes at high, low or intermediate *p*H. Others were more resistant to solvents or heat.

Maxygen has also shuffled genes that make the 20 known human interferons. This time they made 2000 daughter genes—and once again, the results were spectacular. The best interferon produced by the genes was 285,000 times as potent as interferon alpha-2b, which is marketed as a drug, as measured by its ability to protect cultured cells against a mouse virus. It could prove a major money spinner for Maxygen. Sales of interferon alpha-2b, which is used to treat viral diseases and cancer, pull in $600 million each year for Schering-Plough of Berlin. (Source: *New Scientist*, 21 November 1998)

Brain gain

To make a brain, you may need to break a few chromosomes. Cutting and pasting DNA is essential for the development of neurons, a new report suggests, and this might account for some of the brain's unique abilities.

Enzymes that snip chromosomes into pieces and then glue them back together play a part in the development of immune cells. This ability to reshuffle genetic information gives these cells the potential to make billions of different antibodies and immune cells to fight off an ever-changing array of pathogens. But nobody suspected that these cut-and-paste tools played a role in the development of any other tissue.

Fred Alt of the Harvard University Medical School in Boston and his colleagues were studying two of these tools—the genes *XRCC4* and *Lig IV*, which code for proteins that rejoin the severed ends of immune cell chromosomes. As expected, they found that mice lacking either gene had crippled immune systems. But the animals had another problem: they died before birth. "The big question was what was killing them", says Alt. Closer examination revealed that most of their neurons died just after they formed.

This may mean that as neurons develop, their chromosomes have to be rejoined, presumably having first been snipped apart. Alt suggests that cutting and pasting could change gene expression, committing a neuron to a specific fate. The DNA of embryonic neurons might be particularly prone to random breaks, and rely on *XRCC4* and *Lig IV* to heal those breaks. To sort that out, Alt's group is trying to determine which DNA regions are cut during normal neuronal development. (Source: *New Scientist*, 9 January 1999)

Hula-hoops could be the next craze among geneticists

An indigenous new technique based on a molecular hula-hoop will add a powerful tool to geneticists' kit. Its inventors say it could outperform the polymerase chain reaction, the standard technique for identifying and replicating chunks of DNA.

The hula-hoop technique, also known as rolling circle amplification, uses a loop of DNA that sticks to either side of a target sequence and churns out millions of copies of it until there is enough to be detected. The vast number of sequences produced make the test highly sensitive, so it is ideal for tasks such as detecting a few fragments of DNA in criminal evidence, picking out tiny traces of genetically modified foods and screening for mutations.

"Millions of identical copies of the target sequence get reeled off like a long strand of ticker tape", says Mike Evans, the vice-president of drug discovery at Amersham Pharmacia Biotech in Buckinghamshire. Last month, Amersham won a licence to develop the technology commercially with Molecular Staging, a company set up by Yale University in Connecticut, where biochemists David Ward and Paul Lizardi developed and patented the technique.

The scientists found a way to "padlock" strands of DNA to target sequences in genes and chromosomes. An enzyme added to the mixture goes round and round the loop, using it as a template to make multiple copies of the target DNA.

The enzyme used, phi29 polymerase, is different to the Taq polymerase that does the job in PCR—so Amersham believes that rolling circle amplification will not infringe the PCR patents held by Hoffman-La Roche.

Evans says that whole genes can be replicated. And unlike PCR, which churns out millions of discrete copies of the target DNA, the multiple "tickertape" copies produced remain anchored to the target sequence.

So by incorporating fluorescent substances into the tickertape, geneticists can use a microscope to see where the target gene is. What is more, functional genes can be identified in cells by making probes that bind directly to the messenger RNA produced by any active gene.

Evans says that the amplification technique can even be used indirectly to detect when proteins are present in

cells, a feat impossible with PCR. First, antibodies are developed which bind to target proteins. Next, the antibody is attached to a predetermined DNA sequence to which a loop will bind. If the protein is present in a sample, the antibody will capture it. When the loop is added, it will bind to the DNA sequence ready to churn out the usual strand of tickertape copies. (Source: *New Scientist*, 17 April 1999)

From lab to clinic

Even as tissue engineers work to produce whole organs such as bladders and livers, lab-grown versions of more than a dozen different tissues, ranging from skin and cartilage to heart valves and corneas, are either in the clinic or under development. Here is a sample:

- *Approved for clinical use.* The first engineered tissues to hit the market have been skin and cartilage products. In 1997, the United States Food and Drug Administration approved TransCyte, a skin replacement made by Advanced Tissue Sciences Inc. of La Jolla, CA. Consisting of cells from the inner, or dermal, skin layer grown on a biodegradable polymer, TransCyte can serve as a temporary wound cover for some of the more than 30,000 patients hospitalized each year in the United States with second- and third-degree burns. Another skin product, Apligraf, which is made by Organogenesis Inc. of Canton, MA, and consists of both the dermal and epidermal skin layers, was approved last year in the United States and Canada to treat leg ulcers that do not spontaneously heal.

One cartilage product has also won regulatory approval. This is Carticel, made by Genzyme Corp. of Cambridge, MA, to replace damaged knee cartilage. Genzyme takes cartilage-forming cells, called chondrocytes, from cartilage snipped from the patient and grows them in a degradable matrix. The surgeon can then cut out the damaged cartilage and replace it with this new tissue.

- *In clinical trials.* Reprogenesis Inc. in Cambridge, MA, has a different sort of cartilage product in advanced clinical trials. Consisting of chondrocytes growing in a polymer called a hydrogel, which hardens when injected into the body, it is intended to replace defective bladder valves in children with vesicourital reflux, which causes urine to flow from the bladder to the kidney, and in women with urinary stress incontinence, in which patients urinate when they cough or sneeze. Other products in or nearing clinical trials include Dermagraft, from Advanced Tissue Sciences, a variation on TransCyte designed to treat difficult-to-heal foot diabetic ulcers, and Vitrix, a connective tissue product form Organogenesis consisting of fibroblasts and collagen, which helps deep wounds heal without scarring.
- *In the pipeline.* Still in lab studies are a host of additional products. For example, bio-engineer Antonios Mikos of Rice University and his colleagues have recently developed an injectable polypropylene-fumarate copolymer that hardens quickly in the body and provides a surface that guides severed long bones to regenerate in rats and goats. Joseph Vacanti of Harvard Medical School in Boston and his colleagues have recently used a polymer matrix to grow lengths of replacement intestines in rats, which they then attached successfully to the animal's gut. Also in the works are the first lab-grown human cornea, from François Auger's team at Laval University in Quebec City; a portion of the heart's pulmonary valve, grown by paediatric cardiovascular surgeon John Mayer of Harvard Medical School and his colleagues; and soft tissue engineered by David Mooney of the University of Michigan, Ann Arbor, together with colleagues at the Carolinas Medical Center in Charlotte, NC, as a potential replacement for breast tissue removed during mastectomies. (Source: *Science*, vol. 284, 16 April 1999)

Electric DNA

Genes may be able to send electrical signals to one another through a DNA information "superhighway", according to Jacqueline Barton and her colleagues at the California Institute of Technology in Pasadena. The team showed that single electrons can shoot far enough along DNA to influence gene activity.

Last year Barton and her colleagues showed that electrons can pass through short stretches of DNA by hopping between the overlapping electron clouds of adjacent nucleotide bases, the molecular building blocks of DNA.

Together, the disc-shaped electron clouds of each individual base form stacks which serve as an electron-rich pathway for conducting electrical signals.

What surprised the chemists this time, however, was the sheer distance over which a signal could travel. They found that signals could span 60-base chunks of DNA 20 nanometres long, a stretch long enough to code for 20 amino acids. DNA promoters, the molecular "switches" that turn on adjacent genes, are typically this length. The team concluded that in theory, there is no limit to the distance signals could travel along DNA.

But the team also found that specific sequences of DNA bases will stop the signals. These "insulating" regions consist of single or multiple pairings between the two DNA bases adenine (A) and thymine (T). "They serve as electronic hinges in the circuit", Barton says.

The investigators speculate that nature may have engineered these insulators to protect vital genes from electrical damage. In fact, they initially set out to study this type of damage to DNA, which can be caused either by harmful chemical agents called free radicals, or by radiation.

They inflicted this kind of damage on synthetic DNA with ruthenium-based ions which mimic the effects of natural free radicals, which may cause cancer.

Like all oxidising agents, the ruthenium ions lack an electron. In the experiments, they steal one from guanine, the nucleotide base with the weakest hold on its outer-most electron. Barton's team found this happened even if the guanine base was as much as 60 bases away from the ion. But the presence of A and T pairings blocked this

electron transfer. Baton speculates these "electron traps" might prevent the sort of DNA damage that leads to cancer. (Source: *New Scientist*, 13 February 1999)

A Crick in the elbow

If a team of researchers at New York University has its way, engineers may someday turn to DNA as a structural component for building nanometer-scale machines. They describe a robotic arm constructed from two rigid DNA "double crossover" molecules. By changing solution conditions, the sequence linking the two segments can be reversibly switched between two structural states, the B and Z forms, a transition that causes one segment to rotate relative to the other. In order to prove that the predicted motion occurs, the researchers tagged the segments with dyes whose fluorescence varies with the distance between them. "I see this primarily as a prototype. We have demonstrated both that we can make this thing and that we can demonstrate it when it [moves]", says Nadrian Seeman, a professor in the department of chemistry at New York University and senior author on the new work. Seeman adds that because the motion of the DNA arm is relatively large by nanomechanical standards—20 to 60 angstroms—it might be combined with other components capable of smaller movements: "One could imagine this as sort of the elbow or the wrist that could be attached to a robotic finger". The findings are reported in the 14 January 1999 issue of *Nature* (397:144-146, 1999). (Source: *Nature Biotechnology*, vol. 17, February 1999)

Predicting protein function

In an important proof-of-concept experiment for structural genomics, researchers have successfully predicted the biochemical activity of a protein based on data from X-ray crystallography. The availability of complete genome sequences for many organisms has uncovered large numbers of predicted protein sequences with no homology to known proteins. In the new work, researchers have expressed a protein from the hyperthermophile *Methanococcus jannaschii* and solved its crystal structure. Protein crystallization is notoriously chancy, but senior author Sung-Hou Kim explains that studying extremophiles improved the odds of success: "Because these organisms live at very high temperatures, their proteins [often] crystallize much better than their counterparts in other organisms". The structure suggested that the protein is an ATPase, a conclusion that was confirmed by biochemical assays. Kim says that the protein, called MJ0577, is the first the team has attempted to study in this way, and its ATPase domain shares homology with a number of other previously undescribed proteins, highlighting the potential of structural genomics. (Source: *Nature Biotechnology*, vol. 17, February 1999)

E. APPLICATIONS

Pharmaceutical and medical applications

US FDA gives green light to first Cox-2 inhibitor for arthritis

The Food and Drug Administration has given the long-awaited okay to the first of a new class of drugs to control the pain and inflammation from the most common forms of arthritis.

Celecoxib, which will be marketed under the name Celebrex, by Searle, the pharmaceutical arm of Monsanto, and Pfizer, is the first of what are expected to be a wave of Cox-2 inhibitors.

It works by blocking the production of an enzyme, cyclooxygenase-2. The enzyme is believed to trigger the painful swelling that is a trademark of rheumatoid and osteoarthritis, the most common forms of the disease.

The drug was developed by Searle. Cox-2 pain drugs are expected to account for $5 billion in annual sales initially, with the market growing to $25 billion annually by 2007.

Celebrex is a non-steroidal anti-inflammatory drug (NSAID) that prevents the production of prostaglandins by blocking the action of the enzyme cyclooxygenase, or Cox-2. Unlike current drugs to control pain, Celebrex does not block the production of an enzyme, Cox-1, a first cousin of Cox-2. Cox-1 protects the stomach from digestive juices. Without that enzyme patients could theoretically digest their own stomachs, causing ulcers and dangerous gastrointestinal bleeding.

The federal approval is based on clinical tests involving more than 13,000 patients and healthy volunteers. The drug will be widely available to the 40 million United States arthritis victims.

However, the FDA is requiring that the drug carry a warning label outlining the possible risks of ulcers and bleeding in the stomach. Regulators say in their approval statement that more studies in thousands of patients are needed to see if the drug causes fewer side effects than existing painkillers. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 18 January 1999)

"Universal" malaria vaccine unveiled

The world's most advanced malaria vaccine has been developed by US and Indian researchers. If it proves successful in humans, it could lead to the first universally effective preventative therapy for the disease.

The new vaccine is designed to block the parasite at numerous stages of its life cycle. This is not a new idea but has never been attempted so comprehensively before.

Altaf Lal of the Centers for Disease Control in Atlanta, GA, together with colleagues at several US universities and the National Institute of Immunology in Delhi, developed the vaccine after studying how some people developed limited resistance to malaria.

They found that resistant people had a whole repertoire of antibodies against numerous different proteins expressed by the parasite at different stages of its life cycle.

Lal and his team decided to isolate these proteins and combine them into a single, artificial molecule. They built a synthetic gene containing 21 "epitopes"—molecular structures specifically targeted by immune cells—from nine separate proteins. The synthetic gene was then inserted into a virus to make the vaccine.

Earlier versions of this "multivalent" approach have given disappointing results in humans. Lal's vaccine, however, blocks the parasite in more places than ever before. Tests on rabbits showed that it conferred immunity to four separate stages of the malaria parasite. Trials on monkeys are due to start and if these are successful human trials will follow.

Anthony Holder of the National Institute for Medical Research in London says that another key advantage of the vaccine is that the protein structures can be replaced by more potent epitopes whenever these are discovered. It should also be possible to tailor the vaccine to target different strains of the disease. (Source: *Chemistry & Industry*, 1 March 1999)

Poisonous cure

Arsenic, a poison used earlier this century as a treatment for syphilis, has made a comeback as a drug for a rare type of leukaemia.

Raymond Warrell and his colleagues at the Memorial Sloan-Kettering Cancer Center in New York knew Chinese physicians used arsenic to treat patients with acute promyelocytic leukaemia. So they formulated an arsenic compound and gave it to 12 patients who had not responded to other therapies.

In 11 of the patients, the disease went into remission. Six eventually relapsed or died, but five show no signs of cancer. With other treatments, only 10 to 15 per cent of patients achieve complete remission.

Large clinical trials of arsenic are planned for other types of cancer. (Source: *New Scientist*, 14 November 1998)

An affordable therapy for snakebites

Snakebite antidotes could soon be harvested from the yolks of chicken eggs, say Brazilian scientists. An affordable treatment for snakebite victims could save tens of thousands of lives each year in developing countries.

Until now, anti-venom for neutralizing snakebite toxins has been made by injecting horses or sheep with small quantities of venom to produce an immune reaction. Antibodies are then harvested from the animals' blood. However, a single dose of anti-venom costs around $15 and several may be needed to treat each patient. Moreover, the anti-venom contains a mixture of foreign proteins and causes severe allergic reactions in many patients.

The vast majority of the 100,000 deaths from snakebite each year occur in developing countries which cannot afford to keep large stocks of anti-venom. Biotechnology companies are working on a new generation of purer anti-venoms which should be safer (*This Week*, 3 February 1996, p. 18). But this involves altering the structure of the antibody and will make the treatments even more expensive.

Now a team from the State University of Norte Fluminense in Rio de Janeiro is working on a method proposed by researchers at the University of Wisconsin. Claudia Almeida and colleagues have been immunizing hens with small quantities of venom from pit vipers and rattlesnakes and collecting antibodies deposited in the yolk of their eggs.

Tests showed that chicken anti-venom was up to six times as potent as horse antibodies. Equally important, the antibodies in egg yolk are highly concentrated and purer than those in mammalian blood. Laboratories in developing countries would only need relatively simple equipment to produce a safe product.

The Brazilian vets believe that the new method could be cheap enough for use in domestic and farm animals as well as humans. (Source: *New Scientist*, 28 November 1998)

Blind aid

A charitable foundation and the makers of Viagra are joining forces to fight a leading cause of blindness. The Edna McConnell Clark Foundation and the drugs company Pfizer, both based in New York, are launching the International Trachoma Initiative to tackle a bacterial disease of the upper eyelid that infects 146 million people worldwide, mostly in developing countries.

The initiative will encourage preventive measures, such as improved access to water and sanitation, in five African and Asian countries with a high incidence of the disease. By using donations of Pfizer's antibiotic Zithromax, it is hoped that the initiative will eliminate the disease in the countries targeted. (Source: *New Scientist*, 14 November 1998)

Double trouble

Some vaccines now in the pipeline could do more harm than good, say biologists who have been studying the effects of the strange ability of dengue fever virus to cause more severe disease the second time it infects someone.

When a virus or bacterium exists in several strains, infection by one strain usually confers some immunity to the others. This is not true for the dengue fever virus, carried by mosquitoes, which has four main strains. Infection by any strain causes more serious disease if it has been preceded by another.

Immunologists think the initial infection must somehow alter the immune system in a way that weakens the ability of immune cells to resist a subsequent attack. As a result, infection by a second strain of dengue causes more severe illness, and leads to higher levels of the virus in the blood. This in turn probably makes the strain spread more quickly.

To find out how this effect alters the spread of the disease in humans, Neil Ferguson and his colleagues at the University of Oxford created a mathematical model of these infection characteristics. Such characteristics make for extremely complicated disease patterns, Ferguson says. The model also explains the mystery of why outbreaks of dengue fever can crop up suddenly and unexpectedly.

Ferguson also warns that vaccines against dengue, currently under development, could do more harm than good unless they protect against all strains of the disease. Immunizing people against just one or two of the strains using killed viruses would probably increase the virulence of the other strains, he says.

The researchers say similar negative effects could influence future attempts to find effective new vaccines against diseases such as malaria and gonorrhoea. (Source: *New Scientist*, 23 January 1999)

Treatment for liver cirrhosis possible

Chronic alcohol abuse and hepatitis viruses can lead to cirrhosis, a potentially fatal scarring of the liver, generally held to be irreversible. All doctors can do is advise against further drinking and treat complications or, in some cases, offer transplants.

Now researchers at the Hyogo College of Medicine and the Osaka University Medical School have shown that hepatocyte growth factor (HGF) might provide the basis of a potent therapy.

It was already known that HGF makes liver cells multiply, but Toshikazu Nakamura and his colleagues at Osaka think it does much more. They have reported that injections of HGF can improve the condition of livers in rats with fatty liver disease, the first step on an alcoholic's slow march to cirrhosis.

More significantly, a second team from Japan and the US has compelling evidence that HGF can treat cirrhosis itself. Jiro Fujimoto and his colleagues at Hyogo College of Medicine created fatal liver cirrhosis in rats by giving them a liver poison called dimethylnitrosamine (DMN).

They then injected liposomes containing the gene for human HGF into the rats' muscle tissue. Blood levels of HGF rose significantly. Compared with the controls, treated rats showed much less liver tissue damage.

And, remarkably, when tissue samples were imaged they showed a 70 per cent reduction in fibrosis, a key feature of cirrhosis. All 13 rats that received DMN without treatment died within 45 days, with the median survival time being 34 days. In contrast, the 13 rats that received gene therapy had a median survival time of 43 days, and 6 of the 13 lived for more than 50 days. "Taken together with our recent and ongoing HGF studies, I hope that HGF would be useful not only for preventing but also resuscitating liver cirrhosis in humans", says Nakamura. (Source: *New Scientist*, 6 February 1999)

SNPs

CuraGen Corp. announced that is has discovered over 60,000 human genetic variations, or single nucleotide polymorphisms, SNPs, which may be responsible for the development of certain diseases.

The New Haven-based company said it identified the SNPs through its SeqCalling process.

SNPs are of increasing interest to scientists because they are thought to be useful as markers of disease genes and genetic differences which may also determine the response of a patient to drugs.

Currently, there are about 5,000 SNPs in the public domain, with other companies such as Glaxo Wellcome, Celera, Incyte and other companies working to identify SNPs and construct a high-density SNP map—as well as efforts by the Human Genome Project at the NIH.

"SNPs are the most frequent type of genetic variation found in the human genome", said Richard Lifton, MD, Ph.D., Howard Hughes Medical Institute investigator and professor at the Yale University School of Medicine.

"SNPs are found in coding regions of genes and may provide important predictions about responses to drugs and disease predisposition", he added.

The 5,000 publicly known SNPs were derived from random genomic DNA. (Source: *McGraw Hill's Biotechnology Newswatch*, 7 December 1998)

Experimental drugs target genes for ovarian cancer

Over-expression of a specific gene has been linked to ovarian and other cancers, said researchers in Houston and San Francisco.

And at the same time, the research teams have also found at least two investigational drugs that target the over-expressing gene, and have had success in inhibiting the gene and tumour growth in test tube and animal experiments.

These drugs lock on to the ovarian tumour cells because they have much higher protein levels than normal cells—three to 10 times as high.

The findings were reported in the journal *Nature Genetics* by Dr. Gordon Mills, chairman of molecular oncology at the University of Texas' M. D. Anderson Cancer Center, and Joseph Gray of the division of molecular cytometry at the University of California, San Francisco.

Mills said he and his colleagues are using two experimental drugs—wortmannin and LY294002 (a Lilly Pharmaceuticals compound)—in the experiments.

The drugs appear to stop the cancer cells from proliferating by forcing them to undergo apoptosis-programmed cell death.

Ovarian cancer cells also seem to have a higher level of a certain gene, known as PIK3CA, which produces an enzyme PI3-kinase that helps cancer cells grow and invade healthy cells. Mills said the new therapies appear to inhibit PI3-kinase.

That enzyme, Mills said, "plays a major role in cell growth, in the viability of the cell, in its motility or ability to move, in the cell's ability to metastasize or spread, and in the ability to aide the tumours in invasion of other cells".

However, Mills said that by introducing the two anti-cancer drugs the ability to produce the enzyme was inhibited.

"Nothing happened to normal healthy cells. But the tumour cells died. They seemed much more sensitive to the drugs than normal cells", he said.

Mills explained that in pharmacological treatment of cancer the goal is to have the cells which are growing out of control take up the anti-cancer drug more quickly than healthy tissue. Or, another method would be to deliver drugs to which cancer cells are sensitive, but healthy cells are not. So far wortmannin and LY294002 operate in the latter manner.

Mills said he is searching for other compounds, aside from wortmannin and LY294002, to use against PIK3CA.

Human trials with these drugs are expected to begin in 1999. The first studies will explore how toxic the substances are to humans.

Mills said high levels of PIK3CA are not unique to ovarian cancer—they are also found in small-cell lung cancer and possibly others as well. (Source: *McGraw Hill's Biotechnology Newswatch*, 18 January 1999)

Experimental AIDS vaccine uses full virus coat

University of Montana scientists have developed an experimental HIV vaccine that uses the entire protein coat of the HIV virus. They said early results indicate their new approach may produce antibodies against more subtypes of the HIV virus than current HIV vaccine candidates, which use only part of the protein coat.

Other scientists said the discovery is a major step toward development of a broad-use HIV vaccine.

The work is still in a very early stage, with experiments having been done only on mice. But the approach opens up new ways for researchers to develop an effective vaccine that could be given worldwide to prevent the spread of HIV and AIDS, said Jack Nunberg, lead author of the study and director of University of Montana's biotechnology centre. The research was

reported in *Science* magazine. (Extracted from *McGraw Hill's Biotechnology Newswatch*, 18 January 1999)

Experimental DNA hepatitis B vaccine

Results from the first ever successful human trial of a DNA vaccine have been released by Anglo-American biotech firm PowderJect Pharmaceuticals.

Eleven subjects given the company's experimental DNA vaccine against hepatitis B produced enough antibodies to protect them against the potentially fatal liver disease. The vaccine was administered using PowderJect's needle-free injection system, for which the company was granted a European patent.

Schaefer Price, president of US subsidiary PowderJect Vaccines pointed out that the vaccine was delivered in a formulation containing only one-thousandth as much DNA as competing technologies, a factor critical to commercial success.

DNA vaccines are considered to be safer than conventional vaccines because they do not contain the genes responsible for replication or infection, so cannot cause the disease they are intended to prevent. The PowderJect delivery system attaches the DNA to microscopic gold particles, then uses a supersonic blast of helium to shoot these directly into target cells. (Source: *Chemistry & Industry*, 21 December 1998)

RNA vaccine

A new twist on genetic immunization has been reported by researchers at the University of Vienna, Austria. Until recently, most genetic vaccines under development have been based on DNA; however, in a recent report, Christian Mandl and his colleagues have demonstrated the efficacy of an RNA-based vaccine. They showed that mice inoculated with an RNA vaccine developed from the virus that causes tick borne encephalitis (TBE) survive subsequent infection with wild-type TBE virus. After in vitro synthesis of TBE genomic RNA in *Escherichia coli*, the RNA was purified, used to coat gold beads, and inoculated into mice using a gene gun. According to the authors, only 0.1 ng of RNA was sufficient for an infectious dose. In contrast, an attenuated form of the virus created by mutations in a noncoding region of the virus was able to confer protection against live virus at a dose of 5 ng of RNA. In the future, vaccine developers will have to grapple with the thorny issue of whether DNA or RNA is the better vaccine candidate. (Source: *Nature Biotechnology*, vol. 17, January 1999)

Built by bugs

Damaged blood vessels could soon be replaced with artificial tubes built from a polymer tailor-made by bacteria.

Each year, over half a million people worldwide require surgery to replace blocked vessels with healthier tubes taken from elsewhere in the body. But sometimes there are not enough vessels to go around.

David Tirrell of the California Institute of Technology in Pasadena and colleagues have devised a way to build artificial blood vessels that could be implanted in place of a natural vein or artery. The researchers genetically engineered bacteria to produce a polymer similar to elastin, the stretchy protein that lines the walls of blood vessels. This similarity to a real protein is the key to the biomaterial.

The idea is that the protein substitute will be woven to form the basic structure of a blood vessel. Once implanted, layers of endothelial blood vessel cells should grow on the polymer matrix. Cells from a patient could even be grown on the artificial vessels before implantation. Other teams are working on similar ways of creating artificial vessels. Tirrell hopes to test the polymer matrix on animals within a year. (Source: *New Scientist*, 27 March 1999)

Parkinson's patients will be treated with their own neck cells

Transplanting cells from patients' necks into their brains could alleviate Parkinson's disease, say scientists in Spain. Their technique has proved so successful in monkeys that human trials will begin soon.

José Lopez-Barneo of the University of Seville suspected that it might be possible to treat Parkinson's by using glomus cells from a gland called the carotid body, which is located in the neck next to the carotid artery. The gland monitors oxygen levels in the blood. When oxygen levels fall—at high altitudes, for instance—glomus cells in the gland release high levels of dopamine, which alerts the nervous system to step up breathing. Transplanted into the brains of Parkinson's disease patients, glomus cells might restore normal dopamine levels.

In earlier trials of the procedure in rats with a disorder similar to Parkinson's, Lopez-Barneo and his colleagues found evidence that transplants could work, and they have now achieved convincing results in primates. They transplanted glomus cells from two macaque monkeys with a Parkinson's-like disorder into a part of their brains called the putamen. This is the destination for dopamine from the substantia nigra.

Once in place, the transplanted cells seemed to carry on their normal work. Within a few weeks the two monkeys showed marked improvement in mobility and fine motor skills on the side of the body opposite the transplant.

Such treatments could have advantages over other transplant strategies for Parkinson's, such as using foetal cells, which raises ethical problems as well as the possibility of rejection by the immune system.

Four patients with Parkinson's disease will receive glomus cell transplants in Spain over the next few months. (Source: *New Scientist*, 1 May 1999)

Tamoxifen keeps breast cancer from coming back

A new study from the National Surgical Adjuvant Breast and Bowel Project has found that addition of

tamoxifen to the post-surgical regimen following lumpectomy for ductal carcinoma in situ (DCIS) further reduces the risk of breast cancer recurrence.

The current standard of treatment for lumpectomy for EDCIS is a course of radiation. In the women who received that therapy in the NSABP study, breast cancer returned in 13 per cent of the cases, but when tamoxifen was added to the therapy, the breast cancer recurrence was reduced to 8.8 per cent, a 34 per cent decrease, said Dr. Norman Wolmark of Allegheny General Hospital, Pittsburgh, director of the NSABP.

Of the 902 women assigned to the standard therapy of lumpectomy and radiation and placebo pills, there were 103 new cases of breast cancer; of the 902 women who received tamoxifen plus the standard treatment, there were 71 cases of new cancers.

All the women in the study were diagnosed with DCIS, a non-invasive breast cancer involving only the cells lining a duct or milk passage in the breast with no migration to the surrounding breast tissue.

Twenty-five per cent of the 183,000 cases of breast cancer occurring each year are diagnosed as DCIS. DCIS is currently being detected with increasing frequency by mammography. In fact, more than 80 per cent of the women with DCIS in the study were diagnosed with a mammogram, said Wolmark. DCIS is frequently treated with lumpectomy, a procedure in which part of the breast is removed surgically, but the rest is conserved. Breast conservation surgery is routinely followed with a course of radiation therapy.

Wolmark reported that patients who received tamoxifen (manufactured by Zeneca Pharmaceuticals, Wilmington, DE, as Nolvadex) had fewer cases of invasive breast cancer, fewer cases of non-invasive breast cancer, fewer cases of breast cancer recurring in the original breast and fewer cases of cancer occurring in the opposite breast. (Source: *McGraw Hill's Biotechnology Newswatch*, 21 December 1998)

Hepatitis pill cleared by FDA

The US Food & Drug Administration has approved the first pill for the treatment of hepatitis B, a sometimes fatal liver disease that affects about 350 million people worldwide.

The agency also expanded the use of a treatment for hepatitis C that was cleared in June 1998.

The hepatitis B pill, called Epivir, is one of the virus-fighting medications now used in the treatment of AIDS.

The hepatitis B formulation contains a lower dose of the active ingredient—called lamivudine or 3TC—used in many AIDS therapies.

The drug was developed by BioChem Pharma, of Laval, Quebec, which licensed it to Glaxo Wellcome Inc., of Research Triangle Park, NC.

Glaxo Wellcome's Dr. Marc Rubin said, "This once a day oral tablet may provide a convenient and generally well-tolerated treatment option".

"This is a very important approval. It provides an important alternative to currently approved interferon for hepatitis B", said Dr. Heidi Jolson.

Jolson said there are no studies comparing the drugs, so there is no way to tell whether the new treatment works better.

Jolson, who is the director of the division of anti-viral drug products for the FDA, says doctors considering treating hepatitis patients with Epivir should first test them for HIV, the virus that causes AIDs, because giving low doses of the drug could promote the development of resistant strains of AIDS in a person carrying HIV.

Until the approval, the only treatment of hepatitis B was alpha interferon, a drug that must be injected and has a success rate of only about 30 per cent, said Dr. Julie Lehane, a spokeswoman for the American Liver Foundation in New York City.

The FDA also approved an expanded use of combination therapy for hepatitis C. The treatment, called Rebetron Combination Therapy, is two drugs—Intron A, also known as interferon alpha, and ribavirin. In June, the FDA approved the combination, manufactured by the Schering Corp. of Kenilworth, NJ, for patients who had relapsed after treatment with interferon alone. The new FDA approval is for the treatment of patients with chronic hepatitis C who have not been given other drugs. (Source: *McGraw Hill's Biotechnology Newswatch*, 21 December 1998)

Red-handed

Matching blood from the scene of a crime to a suspect may soon be easier, thanks to an antibody test. Researchers say the test is far less time-consuming than DNA tests, and much cheaper.

The test exploits "individual-specific autoantibodies", antibodies which are unique to each person. Scientists at Miragen, a biotech firm in Irvine, CA, have coated paper with strips of proteins to which the antibodies bind. When they place a blood sample on the paper then stain it, a series of purple stripes appears, like a bar code, unique to the person who supplied the sample.

Miragen's test has already been used to track medical samples to avoid mix-ups. But Vicki Thompson at Idaho National Engineering and Environmental Laboratory in Idaho Falls thought the test might be useful in forensic labs. To try it out, she collected blood samples from 10 volunteers and sent them to Wyoming State Crime Laboratory in Cheyenne. At the lab, they were doctored to create 422 samples like those found at crime scenes, for example where blood has been dried on pavements or windscreens, or mixed with petrol.

Thompson and her colleagues analysed the samples using the antibody test and correctly identified 384 of them. In addition, the test was far quicker and cheaper than DNA tests. DNA testing can take weeks, but the new test takes only two hours.

Although the test is not yet accurate enough to stand up in court, it does look promising. (Source: *New Scientist*, 20 February 1999)

A little nutmeg

African herbal folklore has helped put pharmacologists on the track of two compounds that could be used to treat diabetes.

Researchers from Shaman Pharmaceuticals in San Francisco visited Nigeria to investigate the African nutmeg tree, *Pycnanthus angolensis*, which is used by local herbalists to treat diabetes-related conditions such as chronic fungal infections.

From extracts of leaves of *P. angolensis* the researchers isolated two compounds that lowered glucose levels in diabetic mice (*The Journal of Pharmacology and Experimental Therapeutics*, vol. 288, p. 529).

"They have great potential", says team leader Jian Luo. The compounds appear to be more potent than existing treatments for type 2 diabetes, in which people produce insulin but fail to respond to it. The new compounds may enhance the ability of insulin to dispose of glucose. (Source: *New Scientist*, 20 February 1999)

Broad-gauge vaccine for malaria

A team of scientists in the US and India has put together a synthetic protein that it hopes will lead to a malaria vaccine that offers "multiple layers" of immunity. The protein has been shown to stimulate antibody responses to the disease in rabbits, and monkey trials are now under way.

Malaria causes 1.5 million to 3 million deaths a year, mostly in sub-Saharan Africa. The parasite has a complex life cycle—it heads for the liver, where it proliferates and then goes into the blood. That has made it difficult to develop an effective vaccine. Recent efforts have tried to produce immunity to several of the parasite's life stages at once.

The new vaccine is based on information gleaned from looking at antibodies in the blood of Kenyans who have acquired natural immunity to the disease. Altaf Lal of the US Centers for Disease Control and Prevention in Atlanta and colleagues in India assembled 21 gene fragments that code for parasite proteins from various stages of the life cycle. They strung the genes together and inserted them into a virus that can infect insect cells and force them to produce the artificial protein.

Researcher Seyed Hasnain, an immunologist at the National Institute of Immunology, New Delhi, says the work, reported in the 16 February issue of the *Proceedings of the National Academy of Sciences*, "opens up a whole new strategy" for designing a malaria vaccine. Lee Hall of the US National Institute of Allergy and Infectious Diseases agrees, but warns the testing is still at "an early stage" and that other promising candidates have failed clinical tests. (Source: *Science*, vol. 283, 26 February 1999)

Scorpion's venom carries potent toxins to brain tumours

A synthesized peptide derived from the venom of the scorpion—a five-inch long, yellowish creature native to the Holy Land—can seek out, find and cripple brain cancer cells, preventing the spread of disease.

In addition, if that venom-based peptide if fitted with toxins or radioactive particles, it can destroy the cancer cells, giving hope to people who develop the nearly universally fatal gliomas.

Testing of the combination molecules is expected to begin in people later this year, Harald Sontheimer, associate professor of neurobiology at the University of Alabama at Birmingham, said.

"We recently discovered that glioma cells express a glioma specific chloride ion channel not found in other brain cells", Sontheimer said. "This channel appears to facilitate glioma cell invasion by promoting chloride secretion which is essential to induce the shape and volume changes required for glioma cells to migrate through narrow extracellular space in the brain."

The key feature of the peptide is that it only seeks out glial cells, Sontheimer said.

"We discovered that chlorotoxin, a 36 amino acid peptide isolated from *Leiurus quinquestratum* scorpion venom specifically binds to and inhibits the glioma specific chloride ion channel, thereby inhibiting glioma cell migration", he said.

The peptide binds to the receptor and prevents the ion channel from opening and closing. Glial cells are too big to move through the densely packed cells of the brain unless the cells shed water. A chloride ion channel allows the cell to pump fluid out of the cell, letting it shrink and move to areas of the brain where it can grow.

"Experiments we have done shows that the peptide will attach to 98 per cent of these cells", he said.

The laboratory studies were performed upon glioma tissue recovered from patient biopsies.

Sontheimer said because the disease has no cure at present, and a patient's life expectancy is a matter of months after diagnosis, he thinks there will be a rapid processing of Food and Drug Administration paperwork to allow preliminary human testing this year. (Source: *McGraw Hill's Biotechnology Newswatch*, 1 February 1999)

Short therapy stops mothers' AIDS virus from infecting babies

Treating HIV-infected pregnant women with a short course of antiretroviral therapy cuts by 50 per cent the risk of transmitting the AIDS-causing virus to newborns, a new study shows.

Researchers said the study has major implication in developing countries where resources for preventive medicine are limited.

"The results of the study give developing countries a viable option for prevention of transmission of HIV

infection to babies", said Joep Lange, director of the National AIDS Therapy Evaluation Centre at the Academic Medical Centre, Amsterdam.

Lange was one of the people who helped develop the study which was funded by UNAIDS and conducted at five clinics in Uganda, Tanzania and South Africa.

At the 6th conference on Retroviruses and Opportunistic Infections in Chicago, researchers said the four-week treatment with a combination of the drugs zidovudine (AZT, Retrovir, Glaxo Wellcome) and lamivudine (3TC, Epivir, Glaxo Wellcome) before labour and one week after delivery for both mother and child decreases transmission of the disease as effectively as longer, more expensive treatment regimens used in Western nations.

Dr. Joseph Saba, a clinical research specialist at UNAIDS in Geneva, Switzerland, said in the Perinatal Transmission (PETRA) trial 273 pregnant HIV-infected women represented a control group and were given placebo. That group, which represents the natural history of perinatal transmission, recorded a 17.2 per cent transmission rate.

Saba said PETRA involved three treatment arms:

- One group of 359 women was given the AZT-3TC combination for four weeks prior to delivery and then one week post-delivery to mother and to child. The virus was transmitted to 8.6 per cent of these children. That represents a 50 per cent reduction in transmission of the virus, Saba said.
- A second group of 343 women was given the drug combination as labour began and then for a week after delivery. There was a 10.8 per cent transmission of the virus to these newborns—a 37 per cent reduction.
- A third group of 351 women was given the drugs during labour, but not afterwards. There was a 17.7 per cent transmission in that group—not statistically different than the placebo group.

Previous studies have shown that women in Western countries who take drugs to counter AIDS from 20 weeks prior to delivery have a transmission rate of about 8 per cent.

Dr. Biuse Okong, a Ugandan health researcher, said drug costs for African AIDS patients are out of reach for 99 per cent of the people. He said the PETRA study gives governments some handle on what resources are necessary for preventing transmission of AIDS. (Source: *McGraw Hill's Biotechnology Newswatch*, 15 February 1999)

The crop that pumps iron

A strain of genetically modified rice that is rich in iron could help banish anaemia, according to the Japanese scientists who created it.

People whose diets rely heavily on cereals suffer widespread iron deficiency. Some crop varieties draw iron from the soil, but it seldom accumulates in the edible parts of the plant. So Toshihiro Yoshihara of the Electric Power Industry Central Research Institute in Chiba and his colleagues added the gene for an iron-storing protein called ferritin to rice plants, along with a promoter to ensure that the gene is expressed in the grain.

The researchers say that this triples the iron content of the rice. "A meal-size portion would provide 30 to 50 per cent of the daily adult iron requirement," says Yoshihara. (Source: *New Scientist*, 6 March 1999)

Safer blood transfusions

In an effort to make transfusions safer, the main supplier of blood in the US last week began hunting for tiny quantities of HIV and hepatitis C in donations. This test supplements existing ones which only look for antibodies produced in response to infection.

Although antibody tests work well, there is a time lag between infection and the production of detectable levels of antibodies. For HIV, this leaves a "window" of about 21 days in which an infected person's blood may test negative. For hepatitis C, the window can exceed two months. Because of this, about one in every 700,000 units of blood donated in the US carries HIV, and one in every 100,000 carries hepatitis C.

The new test relies on the polymerase chain reaction (PCR) to search for tiny quantities of viral nucleic acids and should reduce these figures to near zero. "We think it is our job to screen as effectively as we can for these viruses," says Richard Davey, chief medical officer for the American Red Cross.

The Red Cross, which collects about half of the 14 million units of blood the US uses each year, began running the test screens in nine of its 37 regional centres. It aims to introduce PCR at all of them by the summer.

GenProbe of San Diego developed the test. It runs on pooled samples taken from 120 donations. If a virus is detected, the tests are run again until the infected sample is found. The test costs about $7 per sample, Davey says, and would increase the costs of supplying blood to hospitals by about 5 per cent.

In 1999, countries in the European Union will begin PCR tests for hepatitis C on pooled blood, but there are no plans to begin similar tests for HIV. (Source: *New Scientist*, 20 March 1999)

Saved from ourselves

A biotechnology company in New Zealand has developed a "curative vaccine" to treat psoriasis, a previously incurable autoimmune skin disease that affects 100 million people worldwide.

Genesis Research and Development in Auckland has completed clinical trials of the vaccine—known as PVAC—in the Philippines. The vaccine is the first to eradicate a disease already in the body rather than preventing it from taking hold.

"PVAC works by blunting the attack of T-lymphocytes directed against the patient's own skin cells", says Jim Watson, who directed the research. In the two-year trial, PVAC cleared symptoms in 50 per cent of patients, which suggests that it only works against one type of psoriasis. (Source: *New Scientist*, 20 March 1999)

Thousands could be saved by platelet receptor blockers

"Super-Aspirins" could save thousands of lives every year, a new study suggests.

Like Aspirin, the drugs—called platelet IIb/IIIa receptor blockers—keep blood platelets from clumping and forming clots in the wake of mild heart attack, chest pain and some heart surgery, according to David F. Kong, of the Duke Clinical Research Institute in Durham, NC, and colleagues.

But the receptor blockers "work far more effectively than Aspirin", said Kong, whose study, which examined the results from 16 clinical trials, was published in *Circulation*, the journal of the American Heart Association.

Patients treated with platelet receptor blockers have their risk of death reduced by 30 per cent, Kong said. The drugs are given intravenously.

Kong added that the study showed that other complications—such as a repeat heart attack or need for a repeat angioplasty or bypass operation—were also reduced. Overall, he said, there were almost 30 fewer deaths or other complications for every 1,000 people treated with platelet receptor blockers.

Physicians have known for years that in severe heart attacks, treatment with a "clot-buster" drug, such as streptokinase or TPA, can save lives. But those drugs are only useful in 30 per cent of patients, Kong said, while the "super-Aspirins" could be prescribed for most heart patients.

The drugs are relatively new. The first, abciximab, is a chimeric monoclonal antibody that was only approved by the Food and Drug Administration in the mid-90s, while two other receptor inhibitors, eptifibatide and tirofiban, got approval early this year. A fourth drug, lamifiban, is still being tested.

Instead of observing patients themselves in a controlled trial, Kong and his colleagues in the United States and New Zealand examined the results of 16 earlier studies, covering all four drugs and involving a total of 32,000 patients.

Kong and his colleagues found the 16 studies through a MEDLINE search; all were randomized, blinded, controlled trials.

The meta-analysis of the results from the 16 studies showed "significant mortality reduction" by the platelet receptor blockers in the first 48 to 96 hours after an ischemic event or an angioplasty, Kong and colleagues write in their paper.

Kong and his colleagues agree that more study is needed. Although they argue that the platelet receptor blockers "clearly show a consistent, substantial and durable benefit", they write that future controlled trials will "undoubtedly refine these results and identify optimal applications for specific agents". (Source: *McGraw Hill's Biotechnology Newswatch*, 4 January 1999)

Four-pronged attack on malaria

The world's most sophisticated vaccine against malaria has passed its first test with flying colours. What makes the new vaccine so unusual is that it combats the parasite which causes malaria, *Plasmodium falciparum*, at each stage of its life cycle.

Tests in rabbits have shown that it works as planned, and trials in monkeys are due to begin. If these are successful, clinical trials in people could begin next year.

In the first stage of malaria infection, mosquitoes inject worm-like sporozoites into their human hosts as they feed on blood. When the sporozoites invade liver cells, they mature into merozoites. In the third stage, merozoites infiltrate red blood cells and mature into egg-like gametocytes. These then burst out of blood cells and are sucked out of infected individuals by feeding mosquitoes. Finally, the gametocytes mature in mosquitoes and produce new sporozoites, which the insect injects next time it feeds.

To develop the vaccine, Altaf Lal of the Centers for Disease Control in Atlanta, GA, which coordinated the development of the vaccine and his colleagues took blood samples from 2,000 children in Kenya. By examining blood cells from the children who had developed immunity to malaria, they discovered several specific regions of parasite proteins most likely to be recognized and attacked by the children's antibodies and white blood cells.

The researchers focused on nine of these proteins, or antigens, from different stages in the parasite's life cycle. They discovered that 21 subsegments of the antigens, called epitopes, promoted even stronger immune responses than the nine complete proteins.

The team then created an artificial gene to manufacture one huge protein containing all the epitopes. By inserting the gene into a baculovirus, which infects insect cells, they generated large quantities of the protein. They then injected the protein into three rabbits.

Since then, the team has conducted further experiments in mice. These show that the vaccine also triggers production of white blood cells which attack the parasite at all four stages of its life cycle.

Lal and his colleagues are now keen to press on with monkey and human trials of the vaccine. "We lose four or five infants per minute to malaria, and with growing resistance of the parasite to existing drugs, there has never been a more desperate need", he says. (Source: *New Scientist*, 20 February 1999)

Laser/drug lung cancer combo gets expanded use label from FDA

A light-activated drug has won Food and Drug Administration approval for expanded use in a system that can help the majority of lung cancer patients.

The laser-plus-drug therapy has been available for early-stage lung cancer, but few people are diagnosed in the early, curable stage of the disease.

The FDA approval expands the use of Photofrin for advanced lung cancer.

In photodynamic therapy—a non-surgical, minimally invasive cancer treatment—patients are injected with a photosensitizing drug such as Photofrin that is selectively absorbed by cancerous tissue. The tissue is then exposed directly to laser light, which can be delivered by an endoscope. Exposure of the light activates the drug, which destroys abnormal cells. Both the drug injection and some endoscopic laser treatments can be performed on an outpatient basis.

Specifically, clearance was granted to market the treatment for the reduction of obstructions and easing of symptoms in patients with certain forms of lung cancer. (Source: *McGraw Hill's Biotechnology Newswatch*, 4 January 1999)

VEGF gene in study

A gene for vascular endothelial growth factor-2 (VEGF-2) will be entering clinical trials for patients with poor circulation in their legs.

The company that will conduct the study, Vascular Genetics Inc. (VGI), or Durham, NC, has received clearance from the Food & Drug Administration (FDA) to initiate Phase I/II human clinical studies to evaluate the safety and activitate the gene to stimulate the growth of blood and lymph vessels in patients suffering from critical limb ischemia.

The VEGF-2 gene, which stimulates angiogenesis, was discovered by Human Genome Sciences, Inc., a major shareholder of VGI.

VGI also plans to submit additional INDs for other indications for this gene therapy product, including coronary artery disease. (Source: *McGraw Hill's' Biotechnology Newswatch*, 4 January 1999)

Boning up on osteoporosis

Researchers have unveiled what could become the first osteoporosis treatment to actually stimulate new bone growth. Researchers from Seattle biotechnology company ZymoGenetics say they have discovered a set of compounds which are showing positive results in animal tests.

The compounds may also help broken bones heal more quickly. And, unlike other bone-growth candidates, they could be administered in a pill rather than by injection.

Healthy bones undergo a continuous process of rebuilding. Cells called osteoclasts break down the old bone, while other cells known as osteoblasts replace it with new material. But in osteoporosis sufferers, the osteoclasts out-perform the osteoblasts. The patient's bones lose mass and become increasingly prone to fractures.

Current treatments act only to slow down the osteoclasts and do nothing to stimulate new bone growth.

Researchers have tried injecting patients with the proteins that the body uses naturally to stimulateosteoblasts, such as parathyroid hormone and bone morphogenic proteins (BMPs). But as Nand Baindur of ZymoGenetics told the meeting, clinical trials have been unsuccessful or inconclusive. He pointed out that the proteins are difficult to manufacture, so any drugs based on them would be expensive. Another disadvantage is that the protein molecules are too large to be taken orally, so must be injected, he said.

Now, by screening tens of thousands of smaller molecules, Baindur's group has found three compounds—two heterocyclic synthetic compounds and one natural product—which can stimulate production of the growth-boosting BMPs. Unlike the proteins themselves, these small-molecule compounds can pass through the gut wall into the bloodstream so they can be taken orally.

"This is the first report of small-molecule drug-like compounds which have been shown to stimulate the formation of new bone in animals", Baindur said.

Osteoporosis is an expensive problem—in the US alone there are between 15 million and 20 million sufferers, and an estimated $3.8 bn/a is spent on treatment. Bone regenerating pills are probably years from the market, but the natural product candidate might have a head start in clinical trials. It is a member of the statin family, some of which are already used to treat heart disease. (Source: *Chemistry & Industry*, 19 April 1999)

New lead found to a possible "insulin pill"

A fungus that grows deep in the African forests near Kinshasa could soon be a pharmacological celebrity. Collected years ago and then analysed by researchers from Merck Research Laboratories in Madrid, Spain, who hoped to find new drugs in rain-forest flora, the fungus, called *Pseudomassaria*, attracted little notice at first. But now another Merck team, led by Bei Zhang and David Moller of the company's Rahway, New Jersey, laboratory, has found that *Pseudomassaria* produces a unique agent that could lead to a new type of antidiabetes pill. Such a treatment would be welcomed by the millions of diabetics who now must inject themselves with insulin or choose from a few orally administered drugs with serious side effects.

The team gave the compound, a small, five-ringed molecule of the quinone family, to mutant mice with symptoms similar to those of patients suffering from adult onset or type 2 diabetes. These include high blood sugar, defects in insulin production, and also a decreased ability of the tissues to respond to insulin. The agent reduced these symptoms in the animals, the researchers found, apparently by tweaking the same cellular receptor that insulin acts on. But, unlike insulin, the fungal compound is not a protein and, thus, could likely withstand the body's potent digestive juices. "This is an insulin mimetic molecule which could become a drug that may be able to be given by mouth", says endrocrinologist Arthur Rubenstein, a diabetes expert at Mount Sinai Hospital in New York. "The potential is enormous."

To find the new compound, Zhang and Moller took advantage of the known activity of the insulin receptor,

which is embedded in the cell membrane. The portion protruding to the exterior spots and attracts insulin molecules, while an inner portion is a kinase enzyme, which responds to insulin's nudging by tacking phosphate groups onto various proteins in the cell. This leads to changes in the activities of those proteins, which in turn allow cells to take up and use the sugar glucose, thereby lowering its blood levels. The insulin-triggered upswing in the receptor's phosphate-adding activity is also useful to researchers hunting for antidiabetes drugs, because they can use it to pinpoint chemicals that mimic insulin's effects.

In tests on cultured cells, the *Pseudomassaria* product, known as L-783,281, stimulated the phosphorylating activity of the insulin receptor by up to 100 times more than other natural compounds tested. And its effects appear to be specific. L-783,281 does not spur the activity of receptors with similar protein-phosphorylating ability, including the receptors for epidermal growth factor, platelet-derived growth factor, and insulin-like growth factor. L-783,281 apparently diffuses through the cell membrane and binds directly to the kinase portion of the insulin receptor, activating it.

Preliminary animal tests with L-783,281 also look promising. The Merck team tested the compound in two mutant mouse strains that have classic diabetes symptoms. In both strains it suppressed the skyrocketing blood sugar levels by up to 50 per cent—comparable to the reduction seen with current oral antidiabetic therapies, Moller says. The compound also reduced the elevated insulin levels seen in one strain, presumably because blood sugar levels dropped, causing the pancreas to lower its insulin production.

If further animal trials confirm that L-783,281 or chemical variants resembling it are both effective in lowering blood sugar concentrations and safe, Merck says clinical trails might be feasible. (Source: *Science*, vol. 284, 7 May 1999)

Glowing beads could help us avoid deadly diseases

Microscopic glass beads wrapped in artificial cell membranes have been made to glow red in the presence of cholera. Chemists in New Mexico say detectors with the beads can sniff out cholera bugs in hospitals and disaster zones before people start falling ill—and that the technology could warn of a biological weapons attack.

As the threat of bioterrorism grows, military researchers are focusing on ways to spot dangerous micro-organisms such as anthrax or cholera bacteria in the field before epidemics break out. Approaches based on DNA sequencing are still slow and relatively insensitive, and the chemicals used in antibody tests have limited shelf life.

To start an infection, bacteria and viruses must capture a receptor molecule on the surface of a cell. The cholera bacterium, for example, secretes a toxin that snags a polysaccharide linked to one of the lipids in cell membranes. Basil Swanson and colleagues at the Los Alamos National Laboratory attached blue and red fluorescent dyes, called fluorophores, to the portion of the cholera toxin receptor that is buried inside the membrane. Then they embedded the modified receptors in an artificial cell membrane coating a glass bead. The membrane is similar in its molecular structure to the membranes that hold living cells together. Such membranes are partially liquid in character, so the receptors float around freely on the surface of the bead.

Blue laser light causes the blue fluorophore to emit light of a slightly redder wavelength. If a red and a blue fluorophore come together, the fluorescence from the blue dye stimulates the red dye to emit red light. But despite their continuous motion, the flurophores rarely meet, Swanson says. However, if a molecule of cholera toxin enters the detector and binds to a receptor, it herds lots of receptors from around the membrane into a cluster. This brings the blue and red fluorophores close together, and the membrane glows red.

The researchers report that by monitoring the red light emitted by the beads, they could determine the amount of cholera toxim present. The US Army has begun testing a breadbox-sized version of the detector, but Swanson says it can be miniaturized further.

The fluorophore detector can be adapted for practically any pathogen. For example, Swanson is now attaching the fluorophores to the receptor for shiga toxin, which is secreted by a harmful form of *Escherichia coli.* (Source: *New Scientist*, 28 November 1998)

Some of the most unlikely organisms have a place in spare-part surgery

Bacteria have been recruited to build artificial blood vessels made from cellulose, the same material that plants use for their cell walls. Surgeons in Germany say the cellulose vessels make good replacements for diseased veins and arteries, and can be used to repair tiny blood vessels during microsurgery—for example, to reattach a severed finger.

The surgeons' little helper is *Acetobacter xylinum*, a bacterium that makes cellulose out of sugars. Bacteria have already been put to work making cellulose sheets, such as those used in the manufacture of loudspeaker diaphragms. Now Dieter Klemm of the University of Jena has found a way to make the bacteria produce narrow cellulose tubes that make good substitutes for blood vessels.

Previous attempts to use cellulose to build artificial blood vessels relied on the clumsy drilling of a duct from a frozen block of cellulose, a "brute force" method that has not taken off. Klemm has developed a culturing technique that allows the bacteria to produce cellulose in the narrow space between a ceramic tube and a core, forming almost ideal artificial blood vessels as little as 1 millimetre in diameter and up to 1.5 centimetres long. The Jena team has found that by using different-sized tubes they can produce vessels tailor-made for particular parts of the body.

Artificial vessels are used to replace arteries or veins damaged in accidents or destroyed—perhaps as a side effect of radiotherapy. Artificial blood vessels made of Teflon are unsuitable for microsurgery because they increase the risk of blood clotting. Instead, vessels have to be taken from some other place in the body.

In tests on rats, the cellulose vessels did not increase the risk of clots and were not rejected. The epithelial cells prevent blood cells from sticking to the wall and causing clots. Local tissue then grows over the outside wall of the cellulose vessel, integrating it almost entirely into the body. (Source: *New Scientist*, 24 February 1999)

Livestock applications

Filter system could clean up fish farming

Land-locked countries could soon be farming marine fish thanks to a new chemical filtration system being proposed by an international coalition of scientists. If it is successful, the system could help alleviate the world's overfishing crisis and also improve the cleanliness of salt-water aquariums.

Leeds University geochemist Michael Krom is leading the project involving researchers from Denmark, Israel and Greece. "Most of the fish eaten in the Western world are marine fish which are either hunted or gathered", he says. "We can not sustain this. Fish farming will eventually have to step in to replace depleted natural fish stocks."

Marine fish farming is almost impossible for land-locked countries, While freshwater fish can be farmed in open lakes and rivers, sea fish have to be housed in artificial tanks. But seawater is a precious commodity in these places, Krom says.

The problem is exacerbated by the toilet habits of the fish, Krom adds. Fish excrete ammonia directly into the water. In artificial tanks, bacteria convert this ammonia into nitrates, which are in turn converted by more bacteria into nitrogen gas, which is removed. But if this process is too successful it can starve other types of nitrate-feeding bacteria found in seawater. These bacteria then turn to digesting sulphates, producing dangerous levels of toxic hydrogen sulphide.

Constantly restocking the tanks with fresh seawater is too expensive. So Krom and his colleagues are designing a filtration system to overcome the problem.

The system uses two separate tanks, one for growing the fish and the other for cleaning the water. The key element in the cleaning tank is an iron oxide cartridge, which mops up any hydrogen sulphide, converting it to iron sulphide and water.

The cartridge will also act as a warning beacon against rising pollution levels. Red iron oxide turns black when it reacts with hydrogen sulphide.

A viable system could also help improve the efficiency of marine aquariums, which have to perform a delicate balancing act between replacing expensive tank water and allowing fish to swim around in increasing levels of ammonia.

The researchers hope to have a working prototype ready in 18 months. A small-scale system due to be built in the Negev desert near Eilat, Israel, will become home to thousands of sea bream. A more advanced plant is planned for Greece. Computer models based on the prototypes will allow the team to fine-tune the system for use in larger fish farms. (Source: *Chemistry & Industry*, 1 March 1999)

No kidding

Goats have been cloned for the first time. Genzyme Transgenics of Framingham, MA, produced a kid called Myra last October, with twins arriving a month later. The goats were created in a similar way to Dolly the sheep, except the cloned cells came from an embryo rather than an adult. The clones all carry a human gene for making antithrombin III, which combats blood clotting and is secreted in their milk. (Source: *New Scientist*, 1 May 1999)

Agricultural applications

Plough to plate

A French seed company is setting up Europe's first scheme for segregating genetically engineered and natural ingredients throughout the food chain.

"It is essential to improve transparency and win the confidence of consumers", says Sofia Ben Tahar, director of biotechnology at Groupe Limagrain, a company based in Chappes.

By the middle of this year, Limagrain aims to have introduced a system for monitoring the fate of plants grown from its seeds. "Each transgenic plant will be coded", says Ben Tahar. Sampling and molecular analysis along the entire food chain—including food processing factories and retail outlets—should allow material grown from the plants to be traced.

This may increase food costs, Ben Tahar admits. For the system to be practical, she adds, a degree of accidental contamination will need to be allowed in products labelled as unmodified. European Union officials are already considering proposals that would allow food to be labelled as "non-transgenic" if the proportion of engineered material among the raw plant materials from which it is manufactured does not exceed a threshold of about 3 per cent.

The segregation of unmodified seeds is already possible, at least in theory. (Source: *New Scientist*, 9 January 1999)

Plant biotech

Monsanto says it will form a joint venture with paper and forestry companies Fletcher Challenge Forests (Auckland), International Paper (Purchase, NY), and Westvaco (New York) to produce and market genetically modified tree seedlings.

The joint venture will focus on tree species that constitute a majority of commercial seedling plantings, beginning with eucalyptus and poplar, *Radiata* and loblolly pine, and sweetgum. Anticipated genetic improvements include herbicide tolerant seedlings, faster-growing trees, and trees with high fibre quality and uniformity, to increase the efficiency of paper production.

The companies will collectively provide $60 million to the joint venture during the next five years; commercialization of products is not anticipated before 2005. (Source: *Chemical Week*, 14 April 1999)

Joint research in proteomics

Dow AgroSciences and Proteome Systems are to carry out joint research into proteomics, an emerging area of biotechnology that involves the study of genes within plants. The goal is to characterize new classes of proteins and identify new enzymes and pathways in the biosynthesis of plant products with improved nutrient content.

According to Keith Williams, founder of Proteome Systems, proteomics research takes the study of genes within plants to a new depth of understanding as the regulation of biosynthetic pathways or the function of proteins in plants is determined. The outcome could result in plants that produce desirable products in a different way or in greater quantity. (Source: *European Chemical News*, 22-28 February 1999)

New plan from industry to preserve Bt crops

This January, the members of an industry coalition consisting of companies that produce *Bacillus thuringiensis* (*Bt*) engineered corn seed outlined an industry version of a unified plan to preserve *Bt* insecticidal toxins and extend the useful lifetime of crop plants that are engineered to produce these insecticides. Monsanto (St. Louis, MO), Novartis Seeds (Greensboro, NC), Pioneer Hi-Bred (Des Moines, IA), and Mycogen-Dow Agro-Science (Midland, MI) are following the advice, given in June last year, of the members of a scientific panel convened by the US Environmental Protection Agency (EPA; Washington, DC) who urged the agency to "require the use of structured refuges" to preserve *Bt*-producing crops (*Nat. Biotechnol.* 15:499, 1997). So far, EPA is not insisting on a refuge set aside, and the industry would prefer to implement a *Bt*-preservation programme on a voluntary basis. Although details are being negotiated, the proposed plan calls for farmers who plant *Bt*-producing corn seed to set aside 20 per cent of their cropland as refuges in which to plant conventional corn, some of which may be treated with conventional insecticides during the growing season. Because the proposal entails uniform refuge set-asides, it could lead to better compliance by growers because what they need to do to meet this *Bt*-preservation strategy is straightforward, according to a spokesperson from Monsanto. (Source: *Nature Biotechnology*, vol. 17, February 1999)

New gene for Novartis

Novartis has developed a new gene which, when inserted into crops, provides tolerance to a class of herbicides known as protoporphyrinogen oxidase inhibitors, or PPOs. The new gene, to be called *Acuron*, will be inserted first in corn for a market introduction in 2003, providing a rival to Monsanto's *Roundup Ready* products.

PPO herbicides kill plants, either crops and weeds, by blocking a key metabolic process, explained Marc Law, who heads the *Acuron* research project. When the *Acuron* gene is inserted into a crop, the metabolic process is no longer blocked by the PPO herbicide, and the plant remains unaffected.

The technology has been found to work on a broad range of crops such as wheat, soya beans, rice, canola, cotton, sorghum and sugar beets. Novartis has filed patents to support and protect this new technology.

PPO herbicides can be both foliar- and root-absorbed and applied within a broad range of timings. According to Novartis, weeds treated by foliar application begin to show the herbicide's effect within 24 hours, with death in two to five days. PPO herbicides can remain effective in the soil for 30-60 days.

The first PPO herbicide will be used both alone and in combination with other Novartis herbicides. Registration for the lead herbicide is in progress with EPA approval anticipated in 2003. (Source: *European Chemical News*, 1-7 March 1999)

Organic farmers can add powerful new tools to their armoury

A bacterium discovered in the roots of Scandinavian crowberry bushes can prevent fungi ruining crops of oats, barley and wheat, according to recent research.

Sprayed onto seeds, the bacterium combats many fungal diseases of barley and oats including the two worst maladies, leaf stripe and net blotch, which can cut yields by up to 20 per cent.

The bacterial treatment took eight years to develop at the Swedish University of Agricultural Sciences in Uppsala. It has been approved in Norway, Sweden and Finland, and was used commercially for the first time this year to treat 60,000 hectares of barley in Sweden. Approval for use throughout the European Union is expected next year.

Live spores of the bacterium *Pseudomonas chlororaphis* are grown in a vat, mixed with edible

rapeseed oil, and sprayed onto the seeds. "The bacteria are dormant until you plant the seeds", says Berndt Gerhardson, head of the team which developed the treatment. "Once in the soil, the bacteria proliferate as the seeds germinate." The bacteria perform as well as chemical fungicides, Gerhardson reports. "There's no difference statistically." Typically, between 98 and 100 per cent of the crops remain healthy.

Unlike many chemical fungicides, the bacterial spray and the seeds treated with it are harmless. The spray has even been approved for use by Sweden's organic farmers, he says.

Meanwhile, Jean-Claude Yvin and his colleagues at the marine biotechnology company Laboratories Goemar in St Malo, France, have discovered that a polysaccharide called β-1,3-glucan, which can be extracted from brown kelp, promises to be effective against a wide range of fungi. Tests by Biotransfer, a company based in Paris, demonstrated that seedlings of wheat, rice, barley and grapes treated with the polysaccharide were resistant to a wide range of fungal diseases.

The glucan does not actually attack the fungal pests, according to tests by Bernard Fritig and his colleagues at the French National Institute for Plant Molecular Biology in Strasbourg. Instead it primes the natural defences of tobacco, tomato and wheat cells to fight off the fungi. Fritig detected several substances on treated plants that are known to combat fungi, including hydrogen peroxide and protective proteins.

This year the glucan made its debut in outdoor trials on wheat fields in the Loire valley. A week after being treated with the glucan spray, researchers deliberately infected the seedlings with *Septoria tritici*, the fungus that causes speckled leaf blotch. After 45 days, only 10 per cent of the leaf area had been attacked by the fungus. A control crop protected with a commercial fungicide fared no better. (Source: *New Scientist*, 5 December 1998)

Transgenic crops

Researchers find themselves in a race to prove the long-term viability of transgenic cotton, corn and potato plants, genetically engineered to produce the insecticidal proteins of the bacterium *Bacillus thuringiensis* (*Bt*), after what experts have dubbed a series of "minor failures" plagued the first three growing seasons.

In Texas, farmers using St. Louis based Monsanto Company's genetically altered cotton plants, referred to as *Bt*-cotton, suffered near-total losses after pests with a high tolerance to the *Bt* toxin produced by the plants moved in and devastated those crops.

Similarly, a planting of *Bt*-cotton in Australia during 1998 required applications of additional pesticides after only one half of the growing season, as the aging plants produced decreasing levels of the toxin.

Brian A. Federici Ph.D., Professor of Entomology and Genetics at the University of California at Riverside argues the problems facing *Bt*-crops are the same that farmers have faced since the general introduction of chemical pesticides after World War II.

The next step, he said, will be the development of more complex toxins. Currently, *Bt*-cotton, corn and potato plants produce only one bug killing protein, resulting in heavy selection pressure that filters the pest populations, and leaves those insects not affected by the *Bt* protein alive to reproduce.

Higher does of toxins produced over the entire growing cycle may help to alleviate the problems experienced by the Australian farmers.

With refuge strategy—planting some percentage of non-*Bt* plants together with the pest-killing variety—farmers are able to diversify the gene pool of the targeted pests by sparing a sub-population of those insects that would otherwise be destroyed.

"The refuge is an area where the insects, regardless of their genetic makeup, survive because there's no toxin", Federici said. "The purpose of the refuge is to dilute out the gene of insects resistant to the toxins."

Federici writes that 4 to 20 per cent of the total crop must consist of non-*Bt* plants, usually planted in rows along the edges, or as blocks within the crop.

Dan Holman, spokesman for Monsanto, said the key to future success for *Bt*-plants lies in compliance with the recommended refuge strategy. Holman suggests that while 5 per cent of the total crop had been considered sufficient for the refuge, currently Monsanto is calling for 10 per cent of the plantings to contain non-*Bt* plants with no additional spraying. He also suggests a 20 per cent refuge for larger scale planting, where insect infestation is considered heavy.

Another concern for *Bt*-plants is their toxicity towards non-targeted species, including the predators that feed on the pests. *Bt* products used to fight caterpillar pests have been found to kill some species of butterfly in the same system. But Federici argues that these costs must be weighed with the benefit of the reduced usage of chemical pesticides.

The pest-killing plant problems have occurred during a period of vocal and most public debate concerning the use of the intellectual property rights of corporations and the life forms they discover. (Source: *McGraw Hill's Biotechnology Newswatch*, 18 January 1999)

Interest in hybrid rice continues to grow

Rice hybrid use is spreading in many sections of Asia, allowing farmers to achieve significantly higher yields. Although initially the adoption and use of hybrid varieties has been slower than expected, the process is now gaining momentum in several nations.

In 1998 Vietnamese rice growers planted 200,000 ha of Chinese hybrids while India grew 150,000 ha of hybrids developed by the Philippines-based International Rice Research Institute (IRRI). In Bangladesh, farmers planted 20,000 ha of hybrid rice introduced from India during the 1998-1999 season. Other countries that have begun to explore the use of rice hybrids are the Philippines, Egypt, Indonesia, Iran, Burma and Sri Lanka.

Rice hybrids take advantage of a process called heterosis, which enables the offspring of two genetically

diverse rice plants to produce more grain than either parent, leading to high yielding irrigated rice varieties. Some commercial hybrids have a yield advantage of 15 per cent (about one ton per hectare) over that of the high-yielding irrigated varieties currently grown in many regions.

In the 1970s, Chinese scientists were the first to demonstrate that hybrids could boost yields, achieving gains of 15 to 20 per cent with the varieties they used. Hybrid rice now grows extensively throughout China. Scientists from China and the IRRI continue their collaboration of their own hybrid research programmes.

Outside China, the spread of hybrid rice has been slower than anticipated, since there are no widespread awareness and promotion campaigns for hybrids, and there is a higher cost for hybrid seed.

An additional concern in southern India is that the available hybrids are all aromatic, which many consumers in other countries favour. In the Philippines, however, non-aromatic rice is preferred, so breeders are in the process of developing the desired trait in a new hybrid.

A number of international and regional organizations have recognized the importance of hybrid rice, and its yield advantages. The Asian Development Bank (ADB) is sponsoring a US$ 1.5 million project called "The Development and Use of Hybrid Rice in Asia", to help spread the use of the technology in Bangladesh, India, Indonesia, Philippines, Sri Lanka and Viet Nam. Additionally the Food and Agriculture Organization (FAO) of the United Nations and the Asian-Pacific Seed Association are involved in projects to develop hybrid rice varieties and to extend their use in more Asian countries.

Updates on the recent situation can be obtained from IRRI, MCPO Box 3127, 1271 Makati City, Philippines. Tel.: +63-2-842-0563. Fax: +63-2-891-1293. E-mail: <d.macintosh@cgiar.org>

Food production and processing

Very precise DNA kit detects GM ingredients in foods

A test launched in March should give teeth to the European Union's strict labelling rules on genetically engineered foods. It detects minute traces of genetically modified soya and maize even in highly processed products, a feat considered impossible until now.

From September last year, retailers in the EU have had to label products containing GM soya or maize. Britain extended the requirement to include menus in restaurants and cafés. But critics complain that the lack of an effective test makes the rules meaningless.

RHM Technology of High Wycombe, Buckinghamshire, says it now has a test that detects traces of GM ingredients even in heavily processed products, such as those containing soya oil or lecithins.

RHM developed the test to allow its parent company, Ranks Hovis McDougall, to monitor the GM content of its own products, which include cakes, sauces, bread and jams. The company will also be able to see if its rivals are failing to label products containing GM ingredients.

The test is also the first to measure accurately what percentage of an ingredient is genetically engineered. That could be important for food inspection agencies. The EU is expected to introduce rules which allow food to be labelled GM-free if less than, say, 2 per cent of the soya or maize within the product is genetically engineered. This will prevent companies being penalized for accidental contamination.

The test registered the correct level of contamination in a loaf deliberately spiked with GM soya flour, says Gordon Wiseman, head of the team that developed it. Soya accounted for 0.67 per cent of the weight of the test loaf, and just 2 per cent of this fraction had been genetically engineered.

Like other tests for GM foods, RHM's detects DNA sequences unique to the transgenic ingredient. These include the cauliflower mosaic virus promoter, the "switch" for activating added genes.

To generate the millions of copies needed for detection and analysis, the DNA fragments in a sample must be multiplied using the polymerase chain reaction (PCR). But food processing degrades DNA. Another problem is that substances in processed food, such as salt, calcium and polysaccharides, block PCR.

To overcome these obstacles, Wiseman's team has designed the test to recognize much smaller fragments of the same genes, which survive processing. The PCR-blocking substances are removed by purifying the DNA with a specialized resin produced by Promega of Madison, Wisconsin.

To establish what percentage of the ingredient is transgenic, Wiseman measures the content of two genes from each sample. One is unique to the genetically engineered ingredient, and one occurs in the transgenic and the natural material. In tests for GM soya, he compares the amount of cauliflower mosaic virus promoter with the amount of the natural soya lectin gene.

Wiseman uses specialized fluorescent probes to monitor accumulation of these gene replicas at each successive cycle of PCR. By comparing the intensity of the glow of the probes for the natural and transgenic sequences, he can calculate the percentage of soya or maize that is genetically engineered. (Source: *New Scientist*, 27 March 1999)

Hope for lactose intolerance sufferers

Relief could be at hand for the millions of people forced to avoid dairy products because the cannot digest lactose, the principal sugar in milk. By putting a rat gene into mice, French researchers have produced mouse milk that has between 50 and 85 per cent less lactose than normal.

If they can do the same with dairy cattle, they hope to mass-produce milk that is low in lactose.

The market for low-lactose milk could be huge. Lactose intolerance affects 70 per cent of the world's

adult population. Most Caucasians are unaffected, but according to the US National Institutes of Health some 90 per cent of Asians suffer from the condition, as do three-quarters of Africans and half the world's Hispanic people. As many as 50 million Americans are estimated to have the condition.

Most symptoms start in childhood, when sufferers cannot produce enough lactase, the gut enzyme which splits lactose into its constituent sugars, glucose and galactose. So lactose builds up, triggering diarrhoea and painful bloating when gut bacteria digest the lactose, creating intestinal gases.

Not surprisingly, people with lactose intolerance shun lactose-rich foods. The alternatives include taking capsules of lactose-degrading enzymes before meals, or adding drops of the appropriate enzymes to milk. Milk low in lactose is available, but to produce it milk must be processed with enzymes or spun in a centrifuge, so it is not cheap.

Jean-Noël, leader of the team which produced the mice at the Strasbourg laboratories of INSERM, the French medical research agency, and his colleagues, introduced a gene into mice that manufactures lactase-phlorizin hydrolase, the rat equivalent of human lactase. To this gene they attached the alpha-lactalbumin promoter, a genetic switch which confined production of the enzyme to the mammary glands of the mice.

Analysis of milk from the mice confirmed that lactose content had dipped by between 50 and 85 per cent. The milk was otherwise identical, and the mice successfully suckled their pups.

Traces of the active enzyme were found on fatty milk globules, but Freund doubts whether such enzymes will pose any hazards. The pig and cattle equivalents of the enzyme are already widely consumed in meat products without ill effects.

Jackie Berning, a lactose intolerance specialist at the University of Colorado, says that if the procedure could be repeated in cows, then lactose-intolerant women, who are at risk of osteoporosis, could benefit from drinking the low-lactose product. (Source: *New Scientist*, 6 February 1999)

Glowing biosensors

DuPont and the University of Delaware (Newark) have devised a method for detecting bacterial toxins, herbicides, and metals in poultry feed by harnessing glowing bacteria. The biosensors can identify toxins such as aflatoxin B1, a known carcinogen, with the help of a hand-held light detector. Researchers created the technology by combining genetic material from the bioluminescent bacterium *Photorhabdus luminescens* with the common bacterium *E. coli*. The technology is especially useful for screening because the biosensors detect classes of contaminants, DuPont says. (Source: *Chemical Week*, 31 March 1999)

Industrial applications

Biodegradable polymers

Bayer subsidiary Wolff Walsrode (Walsrode, Germany) has developed a biodegradable hydroxypropylcelluloselactide thermoplastic for non-film applications. The plastic is produced by copolymerizing cellulose—which provides rigidity but produces non-biodegradable homopolymers—with lactic acid.

Wolff Walsrode has completed initial trials of a lightweight composite material for auto applications, produced by combining its polymer with natural plant fibres. The company is working with Volkswagen to develop a lightweight, rigid version of the material for use in door panels and other auto interior applications.

Wolff Walsrode is hoping that its material's cost—higher than non-biodegradable thermoplastics—will be offset by its biodegradability and low weight, which would ease compliance with a proposed EU ruling requiring automakers to recycle scrapped cars.

Wolff plans to produce about 1,000 mt/year of biodegradable polymer in Europe by 2002. The European biodegradable polymer market is currently 7,000 mt/year. (Source: *Chemical Week*, 11 November 1998)

GM polyester

Scientists at DuPont are working on a new superior type of polyester produced by genetically modified *E.coli* bacteria, called 3GT (three-carbon glycol terephthalate).

The modified bacteria differ in that they have all the enzymes required to convert sugar derived from a corn diet into glycerol, and then turn the glycerol into 3G which they excrete as a milky liquid. If terephthalic acid is then added, the 3G forms 3GT resin and this can be made into a water-resistant fabric. It gives a high-quality polyester that is stretchy, silky and far more "breathable" than the ordinary resin.

DuPont and at least one competitor, Shell, are racing to complete plants designed to manufacture a chemical version of 3GT that they expect to start appearing in garments within a year.

Meanwhile, DuPont has teamed up with Genencor International to patent the biological process for manufacturing 3GT. The project should be at pilot stage by early next year, and it is planned to build a first commercial plant next to a cornfield within the next five years. This should have the capacity to churn out 300,000 litre/day of the resin in its cheaper biological form, sufficient to support a product line.

All liquid effluent is degradable and 3GT can readily undergo methanolysis, so it can be repolymerized and recycled indefinitely (Source: *European Chemical News*, 12-18 April 1999)

Kenaf production

A tropical plant woven into rope for thousands of years in Africa and southern Asia is about to make its debut on Western shores—as an eco-friendly ingredient in car parts and paper.

As its formal name *Hibiscus cannabinus* suggests, kenaf is related to hibiscus, hollyhock, and okra. After 50 years of selection US and European scientists have developed kenaf varieties that can thrive through the changing seasons in warm, temperate latitudes.

Getting ready to cash in on these advances are Kafus Environmental Industries of Vancouver, British Columbia, and Visteon, a Ford Motor Co. division. In February, the two companies broke ground in Elkhart, Indiana, for the first US kenaf factory. (A factory will also start up this summer in Italy.) When combined with polypropylene, kenaf offers a lighter, recyclable alternative to fibreglass in interior car door panels, seat backs, and trim, says Chuck Taylor of Kafus's kenaf-growing facility in Raymondville, Texas.

Plant biologist Ralph W. Hardy, president of the National Agricultural Biotechnology Council in Ithaca, New York, predicts a booming demand for kenaf as car makers move into "bio-based materials". Kenaf is also emerging as an economically feasible substitute for wood pulp now that newsprint prices have shot up. Kafus is going after that market as well, with plans for a mill in Lasara, Texas, that could start turning out an annual 120,000 tons of kenaf-based newsprint by next year. (Source: *Science*, vol. 284, 23 April 1999)

Energy and environmental applications

Textiles waste water treatment

Toray Industries and Toray Engineering have developed a microbial process for treating highly concentrated waste water generated during the caustic treatment of polyester textiles.

The performance of a pilot device (with a capacity of 300 litres), installed at a factory operated by Komatsu Seiren and tested continuously over a period of approximately six months, has confirmed this processing performance and validated the basic technology.

Around 70 per cent of polyester textiles undergo a caustic treatment to improve appearance and texture, in which they are heated with sodium hydroxide to hydrolyse the surface of the material. The waste water from this process contains terephthalic acid and ethylene glycol.

Caustic treatment significantly increases the waste water burden of factories which use the process, to the extent that it accounts for 90 per cent of the total waste water burden at some plants.

Waste water is usually processed by one of two methods: activated sludge treatment as in public sewage treatment, or activated sludge treatment after removal of terephthalic acid, which is precipitated by adding sulphuric acid. The new technology aims to reduce the burden on existing processing equipment through the installation of a bioreactor as a pre-processing device. This also reduced costs by saving space and energy.

Development started with the search for the micro-organisms which are its key point. Two types of naturally occurring micro-organisms, capable of degrading terrphthalic acid under the high temperature and high alkaline conditions of the waste water from the caustic treatment (50° C and pH 9.0) were found.

Subsequently, a third micro-organism, capable of breaking down ethylene glycol under the same conditions, was discovered. This is the first known discovery of micro-organisms capable of breaking down terephthalic acid and ethylene glycol under such conditions.

Over a residence time of five hours, the process eliminated from the waste water more than 99 per cent of the terephthalic acid and more than 70 per cent of the biochemical oxygen demand (BOD). BOD reduction capacity is more than 40 times the capacity offered by the conventional activated sludge process.

The development team now plans to verify processing performance and energy savings on a larger scale and build a full-scale version of the device. The team also hopes to pursue lateral development of the technology to other production processes. (Source: *European Chemical News*, 19-25 April 1999)

Build-up of active BT toxins in soil

Research from New York University indicates that active *Bt* toxins genetically engineered into crops may accumulate in soil. In laboratory experiments, Guenther Stotzky and his colleagues have shown that purified *Bt* toxins, similar to ones found in some lines of transgenic *Bt* crops, do not disappear when added to soil but instead become rapidly bound to clay and humic acid soil particles. The bound *Bt* toxins, unlike free toxins, are not degraded by soil microbes, not do they lose their capacity to kill insects.

Accumulation of active *Bt* toxins in soils could represent a risk to soil ecosystems. Typically, toxins in naturally occurring *Bt* bacteria, and sprays made from them, are not active—they exist in the form of inactive, so-called protoxins. Before they can kill an insect, the protoxins must be dissolved in its gut and cut by protein-digesting enzymes liberating the active toxins. By contrast, the toxin is already in an active form in many *Bt* crops.

Stotzky suggests that active *Bt* toxins might be released to the soil as farmers incorporate plant material into the ground after harvest. Active toxins, which might build up with time, could kill known *Bt*-sensitive soil insects. In addition, a broader range of insects and other organisms may be susceptible to engineered toxins than to toxins from naturally occurring bacteria. Organisms unable to dissolve or cut the protoxin but sensitive to the active toxin would be vulnerable to the engineered active form. Soil-inhabiting insect pests, already exposed to the toxin in their plant-eating phase, may be under continuing pressure to evolve resistance to *Bt*.

Stotzky's results, if they hold true under field conditions, should sound an alarm to regulators and others concerned about risks of genetically engineered crops. To the extent that *Bt* crops containing active toxins are planted in the US, soil organisms may be newly exposed to active *Bt* toxin. Sprays contribute far less active toxin to soil ecosystems because, for the most part, they exist in an inactive form, and unlike the engineered toxins, spray toxins on surfaces of leaves and soil are subject to inactivation by UV light before they are incorporated into soils. Contact: Union of Concerned Scientists, 1616 P Street NW, Suite 310, Washington, DC 20036; tel.: (202) 332-0900; fax: (202) 332-0905; web: www.ucsusa.org (Source: *The Gene Exchange*, Fall/Winter 1998)

Oyster shells used to clean household waste

Discarded oyster shells are to be used to clean household waste water in a prototype plant being built in Japan. The plant will purify water that would otherwise pollute local beaches. "Oyster shells harbour large numbers of anaerobic and aerobic microbes on their surfaces", says Toyokuni Asahina, one of the plant's designers at the Kesennuma City Council. "Dirty water is food for these microbes."

The £3.5 million prototype is being built on Oshima, an island 200 kilometres north of Tokyo where oysters are farmed, and is due to start working in March 2000. About 250 tonnes of shells will be required annually to maintain layers 4 metres deep in the plant's six filtration tanks. Filthy kitchen, bath and laundry water from 265 households will be filtered through the tanks, passing through each in turn.

The more organic waste there is in water, the more oxygen micro-organisms need to break it down. Untreated waste water on Oshima has a biochemical oxygen demand (BOD) of about 200 parts per million, but this should be reduced to less than 20 ppm after the water has been filtered through the oyster shells, Asahina says. Suspended solids will be reduced from 200 ppm to fewer than 50 ppm.

Micro-organisms are already widely used to treat water, but the new plant should be more effective than gravel beds and will provide a use for hundreds of tonnes of waste shells. Another advantage is that oyster shells make acidic waste water alkaline, providing a perfect environment for the micro-organisms. (Source: *New Scientist*, 16 January 1999)

Safer pesticides

Biochips that can spot dangerous pesticides and fungicides at an early stage in their development are being tested by Zeneca, the British agrochemicals manufacturer. The new system should reduce the need for animal testing.

Zeneca expects the biosensor to provide a wealth of data on desirable and undesirable effects. It could show, for example, whether an experimental pesticide would endanger humans, farm animals or plants. It could also help show if the chemicals are dangerous to bees and earthworms, and how effective they are against pests.

Different biosensors are needed to measure effects on different organisms, but all are made in the same way. A chip of glass, nylon or silicon is coated with fragments of genes from one species, each fragment occupying a set position on the surface. The genes used are those known to be switched on when the organism is exposed to high levels of toxic chemicals. In humans, for example, some liver genes become unusually active when exposed to potent toxins.

To test the effect of a particular chemical on the organism, the chemical is added to a culture of the organism's cells. Since messenger RNA (mRNA) is made only when the genes are active, extracts of mRNA can reveal which genes are active. The degree of activity is reflected by the amount of mRNA produced. The mRNA fragments are then labelled with a chemical that fluoresces when exposed to laser light.

Active genes are identified by exposing the biochip to the labelled mRNA. The fragments bind to the corresponding genes on the chip, so the areas with active genes will fluoresce when exposed to laser light.

Zeneca has tested biosensors against known poisons to distinguish between patterns of safe gene activity and patterns that betray unacceptable high levels of toxicity. It has also investigated the effectiveness of its fungicides using a biosensor with all 6,000 genes from bakers' yeast, *Saccharomyces cerevisiae.*

Two thousand human human genes known to be activated by toxic drugs or pesticides are being tested to find the best indicators among them. (Source: *New Scientist*, 13 March 1999)

H. pylori in water pipes

The slime that coats water pipes could be a haven for bacteria that cause stomach ulcers and gastric cancer, according to researchers at Robert Gordon University in Aberdeen.

Helicobacter pylori has been implicated in a wide variety of gastrointestinal diseases, from stomach inflammation to ulcers and even cancer. In developed countries, half of people over 50 years old carry the bacterium in their guts, and in developing countries an even larger proportion of people are affected. Several animals may harbour the micro-organism, but so far no one has found a reservoir in the environment.

Donald Reid and his colleagues in Scotland thought that *H. pylori* might thrive in biofilms. These form when micro-organisms colonize surfaces such as the insides of water pipes, often surrounding themselves with a sticky protective film. "Biofilms occur naturally in all water distribution systems", says Reid.

The researchers grew a biofilm inside stainless steel pipes in the laboratory and inoculated it with *H. pylori.* Even after unchlorinated water was flushed though the pipes for 192 hours, *H. pylori* still infested the biofilm.

"Anywhere there is a biofilm, there is a potential haven for *Helicobacter pylori*", says Reid. This is

particularly likely in unchlorinated private wells and water supplies in developing countries. Although most public supplies in developed countries are chlorinated, Reid believes the bacteria could still survive inside the biofilm. (Source: *New Scientist*, 3 April 1999)

Superbug survives radiation

A can of spoiled meat and nuclear waste may appear to have little in common, but the microbe *Deinococcus radiodurans* finds both environments rather cosy. Scientists hope this organism's ability to withstand massive doses of radiation will make it a useful tool for toxic-site remediation.

Although scientists now find it in many different soil and water sites around the world, *D. radiodurans* was not identified until 1956. It was isolated from a can of ground beef that had been radiation sterilized but had spoiled nonetheless. Perhaps because it can efficiently repair radiation breakage of its own DNA, *D. radiodurans* can endure 1.5 million rads of radiation, a dose 3,000 times higher than would kill organisms from microbes to humans. Scientists are unsure how this resistance evolved, although they suspect it may be a side effect of the microbe's ability to survive periods of severe dehydration, which also fragments DNA.

Recognition of *D. radiodurans'* resistance to radiation led DOE Microbial Genome Program (MGP) managers to believe the microbe could be useful in cleaning up mixed-waste sites contaminated with toxic chemicals as well as radiation. They began to fund projects to decipher the microbe's genome and alter it to detoxify the most common chemical contaminants at these sites. Such detoxification functions might include concentrating heavy metals and breaking down organic solvents such as trichlorethylene.

Clean-up of toxic sites created by improper disposal of nuclear wastes presents a massive global challenge requiring innovative remediation approaches. In *Nature Biotechnology* (vol. 16, October 1998), Michael Daly and Kenneth Minton (Uniformed Services University for the Health Sciences in Bethesda, Maryland) described a first step toward enhancing the *D. radiodurans* genome to make it valuable for toxic-site clean-up.

Daly and Minton reported successfully altering the microbe's genome. This was accomplished by first fusing a gene encoding toluene dioxygenase (an enzyme that degrades the organic contaminant toluene) to a *D. radiodurans* promoter (a site that activates the gene). This DNA was then inserted into one of the bacterium's chromosomes. The resulting recombinant bacterium is capable of degrading toluene and other organic compounds in a high-radiation environment. It also is tolerant of toluene and trichloroethylene's solvent effects at levels exceeding those of many radioactive waste sites. (Extracted from Human Genome News 10 (1-2), February 1999.

New sensor for pollution detection

Glowing yeast cells could soon tell drugs and water companies if there are potential carcinogens in their products. The genetically modified cells are part of a new type of sensor that could revolutionize the detection and identification of pollutants.

With the help of a gene from a jellyfish, researchers in Britain created yeast cells that fluoresce bright green when exposed to substances that damage DNA. "The more damage, the brighter the glow", says Richard Walmsley, head of the team that developed the device at the University of Manchester Institute of Science and Technology. The monitor could spell the end for the Ames test, the standard procedure for identifying substances that alter DNA and thus might be carcinogenic.

Walmsley and his colleagues expect water utilities to use their device as a pollution sensor. For the first time, impending legislation in the European Union will force all water companies to monitor supplies for cancer-causing pollutants. And drugs firms could use the monitor to weed out drugs that might later cause tumours.

The monitor was designed to over the limitations of the Ames test, developed in 1973 by Bruce Ames, a biochemist at the University of California at Berkeley.

The new invention gives a result in as little as four hours and relies on yeast cells, which are more closely related to mammalian cells. The yeast test also shows up subtle but repairable damage to DNA. The researchers equipped the yeast with a gene for a green fluorescent protein from the jellyfish *Aequoria victoria*. The protein glows bright green when bathed in blue laser light. They engineered the yeast so that the jellyfish gene would be turned on at the same time as *RAD54*, a gene switched on whenever yeast DNA needs repairing.

In this way, the brightness of the green light reflects the amount of repair under way in the yeast cells. The brightness, as measured by a fluorescence detector, rises rapidly above background levels when the yeast cells are exposed to a mutagen. To test the system, Walmsley and his colleagues exposed the cells to methyl methane sulphonate, a known mutagen. The amount of fluorescence doubled in just four hours. (Source: *New Scientist*, 20 February 1999)

Hope for MTBE users

Researchers at the University of California Davis have discovered a microscopic organism, PMI, that rapidly eliminates MTBE pollutants from soil and groundwater, said Kate Skow, a microbiologist at UC Davis and leader of the research.

Field tests on the UC Davis microbes, sponsored by the Oxygenated Fuels Association—an international trade association established to advance the use of oxygenated fuel additives—and the US Navy, were scheduled in Port

Hueneme, CA. Tests on a similar strain are under way in central CA, sponsored by Shell Oil.

MTBE is used to boost octane in gasoline, promote cleaner burning fuel and reduce harmful vehicle exhaust emissions. However, California's recent ban on the additive was based on the result of two studies which, the state says, throw doubt on its usefulness in reducing harmful emissions and reinforce fear of MTBE's potential to contaminate groundwater.

MTBE consumption in 1998 is estimated at around 2.4 m tonne/year. The largest market is the US. MTBE has been under attack for years but recently the contamination of groundwater in CA has become an issue in a state where water supplies are scarce.

MTBE is highly soluble in water and transfers readily from gasoline leaks from underground storage tanks, pipelines and other components of the gasoline distribution system. (Source: *European Chemical News*, 12-18 April 1999)

Crystal clean-up

Nanoscale crystals could become powerful agents against chemical and biological warfare. Researchers from Kansas State University say that tiny crystalline particles of metal oxides can be used to decontaminate battlefields and buildings and could also be incorporated in protective clothing and skin cream.

The researchers, led by Kenneth Klabunde, have shown that nanocrystals of magnesium oxide, coated with halogens, are highly effective at adsorbing and destroying chemical warfare agents including HD (mustard gas) and the nerve agents VX and GD.

The group also found certain formulations of nanoparticles killed several bacterial spores including *Bacillus cereus* and *B. globigli*, which are safe mimics of anthrax used in preliminary laboratory tests.

Because of their tiny size (4-9 nm), the nanoparticles have a huge surface area—around 400 m^2/g—and can be spread over vast areas. Fifteen litres of the powder could cover 360 football fields. "Such a surface could theoretically detoxify around three pounds of HD mustard", Klabunde said. He hopes to develop a single formulation that works against all chemical and biological weapons. However, he pointed out that, as with antibiotic medicines, such formulations will probably also kill "good" bacteria. "They need to be applied where needed and not indiscriminately", he said. The reactive nanoparticles degrade when exposed to the air for prolonged periods. (Source: *Chemisty & Industry*, 19 April 1999)

Decontamination of toxic residue

As the United Nations pursues its crusade against bioweapons in Iran, scientists are turning their attention to mopping up human-made plagues before they can do any harm. US government researchers are designing a foam that they hope will deactivate everything from the deadliest bugs to nerve gas without leaving a toxic residue behind.

It is useless to clean up one hazard if you leave another in its place, says William Earl, a physical chemist at Los Alamos National Laboratory who coordinates government decontamination research. So chemists at the Sandia National laboratory near Albuquerque, New Mexico, are perfecting a cocktail of hydrogen peroxide, baking soda and other household ingredients that will oxidize nerve agents and break open bacteria. The mixture is blended with a foam for easy application to walls and furniture. Tests with harmless bacteria and non-toxic chemicals have been successful, and the Army plans to test the foam on VX, mustard gas and microbes such as anthrax.

If the foam proves effective, researchers will still have to optimize it for different surfaces, Earl says. Decontaminants that work in a test tube may not work on a desk or a carpet. Greg Thomas, a project manager at Sandia's Livermore branch in CA, says the San Francisco airport is considering equipping its new terminal with the foam if the tests are successful. (Source: *New Scientist*, 28 November 1998)

Biohazards

Doctors warn of weapons threat

Biological weapons designed for "ethnic cleansing" could be used within ten years, doctors have warned. The British Medical Association (BMA) says that advances in genetic engineering techniques will soon make it possible to create weapons that can target specific ethnic groups.

A new report by BMA doctors warns that genetic information is already being used to enhance biological weapons. The doctors are urging biotech researchers to be vigilant to prevent their work being put to such use.

The BMA found that genetic research is already being used to improve the efficiency of biological weapons, for example by increasing antibiotic resistance. Within five to ten years it will probably be possible to design weapons that exploit the subtle genetic differences between ethnic groups, Nathanson warned.

Biological warfare research can be almost impossible to distinguish from legitimate medical study. A crucial part of the human genome project involves identifying the genetic differences between ethnic groups to discover why certain groups are susceptible to particular illnesses.

The BMA report also calls for a strengthening of the 1972 Biological and Toxic Weapons Convention, the only disarmament treaty not to include monitoring or enforcement provisions. The European Union launched negotiations on the issue at the Geneva Conference on Disarmament last month.

The human genome is deteriorating, UK biologists have shown. Mutations in the the human genetic sequence with an adverse effect on fitness occur at least 1.5 times per generation—a high enough rate to threaten long-term survival if the effects were to combine (*Nature*, 1999, 397, 344). (Source: *Chemistry & Industry*, 1 February 1999)

F. PATENTS AND INTELLECTUAL PROPERTY RIGHTS

Workshop on Intellectual Property Rights suggests need for change in existing regimes

Prompted by the global interest in the intellectual property regime a workshop was convened in New Delhi, in January 1999, to discuss and provide expert thinking on the conflicts and complementarities between the Convention on Biological Diversity (CBD) and the Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPs) of the World Trade Organization (WTO).

The statements and recommended actions reflect the range of views expressed at the workshop and are offered as suggestions to the international bodies dealing with these issues.

In summary, there was strong support for the three objectives of the CBD; conservation of biological diversity, sustainable use of its components, and the fair and equitable sharing of the benefits arising from such use. Participants also recognized the immense contribution of traditional knowledge and practices of local and indigenous communities for conservation, and reaffirmed the need for the effective maintenance of such knowledge systems. In relation to the TRIPs Agreement, participants recognized that the objective of the Agreement—to protect Intellectual Property Rights (IPRs)—should provide benefits to both producers and users of technological knowledge in a manner conducive to social and economic reality.

Concern was expressed that the current TRIPs Agreement fails to adequately address a number of issues central to the achievement of the objectives of the CBD, and, furthermore, pose a significant threat to the conservation of biodiversity. Additionally, TRIPs does not address a range of equity issues (including intergenerational equity), and it renders difficult both access to genetic resources and the fair sharing of benefits arising from their use. Perhaps more seriously, the workshop participants noted, that the Agreement fails to recognize and protect traditional systems of knowledge that are needed to meet the objectives of the CBD fully. It was concluded that there is, therefore, a need to amend existing regimes and/or develop alternative regimes to address these concerns. (Source: *Diversity*, vol. 15, No. 2, 1999)

Indian patents don't impress

The Indian subsidiaries of such major pharmaceutical firms as Glaxo (London), Novartis (Basel) and SmithKline Beecham (London) saw their share prices soar on the Bombay market following the Government's announcement at the end of November 1998 that it would introduce new patent laws. The new laws, to be introduced before April 1999, will allow companies to hold exclusive marketing rights to a product. However, these marketing rights do not prevent the production and sale of generic versions of drugs in India, and are only the minimum requirement set down by the World Trade Organization (WTO, Geneva), of which India became a member in 1995. The US and the European Union have pushed India to allow product patents, which would prevent copying of drugs for 20 years. However, according to WTO rules, India does not have to implement this until 2005. The US drug company Pfizer (New York), has said it would rather wait until India provides product patents before launching its latest drugs in India. (Source: *Nature Biotechnology*, vol. 17, January 1999)

European Parliament approves controversial biotechnology patent legislation

After 10 years of intense debate, the European Parliament approved highly controversial European patent legislation for biotechnology on 12 May 1998 at a meeting in Brussels. The "Biotech Patents Directive" affirms the right of Europeans to make property rights claims on living organisms and human genes, proteins and cells.

Patent offices in most European countries had already been awarding patents on human gene sequences, but the laws concerning transgenic plants had not been clear. Hereafter, a patent will cover all plant varieties with a particular characteristic. European member States are required to enact the directive as national legislation

within two years. This would bring European practice on biotechnology in accordance with the policy of the US, Japan and other industrialized countries.

Environmentalists and other opponents of this legislation believe that, among other threats, the legislation will stifle academic research.

Most troubling to the many non-governmental organizations representing agricultural, medical and religious interests that had fought to stave off the "Life Patents Directive", was that the version passed by the European Parliament was irtually identical to the text it deemed "unethical" and rejected in 1995. In the interim, the European Parliament made more than 60 amendments to the draft Directive attempting to mitigate some of the more inflammatory charges such as "biopiracy" and the misappropriation and privatization of biological materials from third parties without prior informed consent. None of those amendments appeared in the final Directive following what non-governmental organizations charge was "the largest lobby campaign of the multinational biotech industries." (Source: *Diversity*, vol. 14, No. 1 & 2, 1998)

G. Books, Journals, Reviews and Bioinformatics

Geographic Information System

Preliminary results from an international research initiative confirm the usefulness of the Geographic Information System (GIS) technology in locating genetic diversity of the cultivated peanut. Experts believe the GIS approach could be useful in identifying areas of high diversity and risk of genetic erosion in cultivated species. The technology is under investigation through a collaborative project involving the International Center for Tropical Agriculture (CIAT), the International Plant Genetic Resources Institute (IPGRI-Americas) and the US Department of Agriculture's (USDA) National Germplasm Resources Laboratory. The project's activities include (a) studies of the relationship of the human and physical environment with the distribution of cultivated peanut diversity in Ecuador using morphological characterization of peanut landraces, socio-economic and environmental data, and satellite imagery; and (b) identifying and using variables that correlated most closely with the distribution of peanut diversity to predict the distribution of peanut diversity in Guatemala where germplasm collecting is still under way. (Source: *Diversity*, vol. 14, No. 1 & 2, 1998)

Biocommerce introduced

Fiz Karlsruhe, a German-based information centre for science and technology, has introduced a business database called Biocommerce on biotechnology and biological sciences worldwide. The database, with over 170,000 records, has been produced by the UK's Biocommerce Data.

Biocommerce is said to cover all aspects of biotechnology, including genetics, immunology, microbiology and molecular biology, as well as its application in areas such as chemicals, energy, health care and waste treatment. It also includes profiles on more than 2,500 companies and institutions in biotechnology with more than 11,000 contact names. (Source: *European Chemical News*, 8-14 March 1999)

New edition of the European Biotechnology Directory

The newly published 1999 edition of the European Biotechnology Directory reveals the fastest growth in the European biotech sector is coming from Germany.

Dr. Gerd Romanowski of the German Association of Biotechnology Industries in one of many articles included in the directory writes that in the last four years, the situation in Germany has changed dramatically. According to Dr. Romanowski, the BioRegio competition, initiated by the German Federal Research Ministry, marked the starting point of this boom, which has led to the whole of Germany being caught up in a veritable biotechnology euphoria. In this process seventeen bioregions have been formed competing for prizes and funds. In total, the German Federal Research Ministry has spent close to DM 1,000 million in 1998 to support biotechnology in Germany.

The German biotechnology industry is well on the way towards catching up with the leading groups of highly innovative biotechnology landscapes in the US and UK, and comprises all the fields of the value added chain from the research idea all the way up to the approved product. The prospect of collaborations with international companies is, in particular, a guarantee that German companies will remain competitive.

The macroeconomic effects of this German boom in biotechnology are already manifesting themselves today. The small and medium-sized companies are already employing approximately 11,000 people. Furthermore, the added value of biotechnology is manifested by the fact that 23 large companies that are conducting research and are involved in the production in the life sciences sector in Germany are already generating sales of around DM 3,000 million today with biotechnology products. In total, the sales of German big, medium-sized and small companies with biotechnology activities accounted for DM 4,400 million in 1997.

The new 1999 edition of The European Biotechnology Directory includes an additional 14 topical articles on current issues and developments in biotechnology, such as progress in plant biotechnology, and implementation of the biotech patenting directive—as well as profiles of a number of European biotech country markets.

Listing over 1,400 organizations involved in biotechnology and containing 3,200 senior contact names, this invaluable reference source includes detailed profiles of the activities of nearly 1,000 biotechnology companies in 19 countries and also includes financial information, staff numbers and year founded, as well as e-mail addresses and World Wide Web URL's.

To order a copy of The European Biotechnology Directory '99 (£215/$355) or to request information or samples of any BCD or PJB products, please contact: BioCommerce Data Ltd., 18/20 Hill Rise, Richmond, Surry TW10 6UA. Tel.: 0181 332 4660, Fax: 0181 332 4666; e-mail: biocom@dial.pipex.com (Source: *Press Release*, 28 May 1999)

Bioresources and Biotechnology: Policy Concerns for the Asian Region

Edited by Suman Sahai, pp. 174, IAR.200, published by Gene Campaign, L.235/A, Sainik Farms Khanpur, New Delhi 110 062, India.

Developing countries have most of the world's bioresources. It is their communities that have protected these resources over millennia. However, lack of proactive policy deprives these communities from reaping their rightful benefits.

Biotechnology has emerged as a powerful technology of the present time, which with appropriate planning, could contribute to significant economic growth for the developing world. The need to define a common agenda and evolve clear-cut policies in this respect prompted Gene Campaign (a non-governmental organization pioneering the struggle for farmers' and community rights) to organize a seminar on Bioresources and Biotechnology Policy for the Asian Region (meeting of experts from India, Bangladesh, Nepal, Sri Lanka, Malaysia and the Philippines).

The book highlights special problems such as the intellectual property rights (IPRs) which confront the developing countries with regard to bioresources and biotechnology, and suggests an effective strategy. The recommendations emphasize the requirement for common capacity-building and mutually accessible databases, resource-sharing and easy transfer of technology.

New global studies reveal unabated loss of biological diversity

The 1997 World Conservation Union (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, including about 29 per cent of the 16,000 identified species in the US, 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 modeled 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 IUCN, in cooperation with the Smithsonian Institution, the World Wildlife Fund (WWF), the Nature Conservancy, the Royal Botanic Gardens at Kew and Edinburgh, and 10 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 CB3 ODL, UK. Tel.: +44-1223-277894; Fax: +44-1223-277175; e-mail: <IUCN-psu@wcnc.org.uk> or: Ricardo Bayon, IUCN-UD. Tel.: +1-202-797-5454; Fax: +1-202-797-5461; e-mail: <rbayon@iucnus.org> (Source: *Diversity*, vol. 14, No. 1 & 2, 1998)

Library of Traditional Knowledge and Biodiversity CD-ROM

Is a collection of documents and databases related to biological diversity and the implementation of article 8 (j) of the Convention on Biological Diversity, a collaborative project between the Indigenous Biodiversity Information Network (IBIN), the International Conservation Networking System (ICONS), the Indigenous Knowledge Program (IKP) and The World Conservation Union (IUCN). This CD-ROM contains a preliminary version of a document library related to indigenous knowledge and biodiversity, a copy of the ICONS conservation data management system, an extensive database of information related to conservation and biodiversity, and software utilities to help you use the resources on this disk. Distribution is free for non-governmental organizations. Indigenous Knowledge and Biodiversity CD-ROM, ICONS, IBIN, IUCN, IKP, 1998. For more information contact: Preston Hardison, ICONS Project, 8226-4 1st Avenue NE, Seattle, WA 98115, USA. Fax: (1-206) 527-0119; e-mail: pdh@u.washington.edu pdh@igc.apc.org; web: http://www.iucn.org/icons (Source: *Seedling*, March 1999)

World Hunger: Twelve Myths

The authors of *World Hunger: Twelve Myths*, are the founders of the US-based Institute for Food and Development Policy, well known for its ground-breaking work on food and development issues. The book is a fully revised and updated version of *World Hunger*, which was published 12 years ago. In it, the authors counter the myths about hunger which are commonly accepted. The book

tears apart, in data-laden chapters and thorough analysis, the misconceptions people have about the roots of hunger. It also offers ideas for pro-active initiatives.

Frances Moore Lappé, Joseph Collins, and Peter Rosset, *World Hunger: Twelve Myths*, Second Edition fully revised and updated, Food First Books, Oakland, 1998, 270 pp., ISBN 0-8021-3591-9. Priced at US$ 13,00. Order from: Food First Books, 398 60th Street, Oakland, CA 94618, USA. Fax: (1-510) 654 45 51; e-mail: foodfirst@igc.apc.org (Source: *Seedling*, March 1999).

The State of the World's Plant Genetic Resources for Food and Agriculture

The Food and Agriculture Organization of the United Nations (FAO) has just released the formal edition of *The State of the World's Plant Genetic Resources for Food and Agriculture*, approved at the Fourth International Technical Conference on Plant Genetic Resources, in Leipzig, Germany, in June 1996. Includes FAO's report on the State of the World's Plant Genetic Resources for Food and Agriculture, 154 national reports, and the results of 11 subregional and regional preparatory meetings, plus various other background materials. The report covers the state of diversity in the world's agrecosystems, *in situ* management, *ex situ* conservation, utilization of genetic resources, national programmes, training needs and legislation, international collaboration, and the issues of access to plant genetic resources, and the sharing of the benefits derived from their use and the realization of farmers' rights. The numerous annexes and appendices are nearly as useful as the Report itself. Perhaps the best aspect of the Report is that it exposes clearly some of the fundamental problems facing our food production systems: the unabated erosion of plant biodiversity, both in the field and in seed banks, the insufficiency of *ex situ* conservation as the system to counter the genetic erosion produced by the industrialization of agriculture, and genetic uniformity in our crops. The book comes with a CD-ROM which includes many of the book's most interesting contents.

FAO, The State of the World's Plant Genetic Resources for Food and Agriculture, Food and Agriculture Organization of the United Nations, Rome, 1998, 510 pp. ISBN 92-5-104073-7. Available from: Plant Production and Protection Division, FAO, Viale delle Caracalla, 00100 Rome, Italy. Fax: (39-6) 5705 55 33; e-mail: ICPPGR@FAO.ORG (Source: *Seedling*, March 1999)

Altered Genes

Altered Genes, is the first book that presents a critical perspective of the many important issues raised by modern biotechnology from an Australian perspective. The book is structured in three parts. "Setting the agenda" deals with the more general issues on the corporate agenda vis-à-vis the public and regulatory agencies, biopiracy and the flaws of the scientific paradigm behind genetic engineering. "Bioethics, eugenics and risk" deals mainly with the implications of genetic engineering on human beings, including the increased erosion of women's control over procreation. Finally, "Molecular farming, novel food and campaigning" goes deeper into agricultural production and novel foods. The book starts with an excellent introduction by geneticist Dr. David Suzuki.

R. Hindsmarsh, G. Lawrence and J. Norton (eds.), *Altered Genes, Reconstructing Nature: the Debate*, Allen & Unwin, St. Leonards, 1998, 228 pp., ISBN 1-86448-795-X. Order from: Allen & Unwin, 9 Atchison Street, St. Leonards NSW 1590, Australia. Fax: (61-2) 8425 01 00; e-mail: frontdesk@allen-unwin.com.au web: http://www.allenunwin.com.au (Source: *Seedling*, March 1999)

Biological Diversity in Namibia

Biological Diversity in Namibia: A Country Study, results from a three year study on the biological diversity of Namibia. It represents a very significant achievement in terms of bringing together previously fragmented information held by biologists, ecologists and other specialists. Includes an analysis of the economic values of Namibia's biodiversity, as well as the legal framework for biodiversity conservation and sustainable use. Beautiful, generously illustrated, and data-filled.

P. Barnard (ed.), *Biological Diversity in Nambia: A Country Study*, Namibian Biodiversity Task Force, Windhoek, 1998, 322 pp., ISBN 0-86976-436-5. Order from: Namibian Biodiversity Task Force, Directorate of Environmental Affairs, Windhoek. Tel.: (264-61) 24 90 15; Fax: (261-61) 24 03 39; e-mail: pb@ dea.met.gov.na (Source: *Seedling*, March 1999)

Pakistan Journal of Biological Sciences

A quarterly publication, published by the Capricorn Publications. This is the first Asian scientific journal, which is also available on the Internet (http://www.pjbs.org). It consists of regional editors from developed countries and a group of technical editors that are competent research scientists in their respective fields. This is the most regular journal published in Pakistan. More than three thousand visitors visit the journal's web site per month from all over the world. It shows the acceptability and recognition from the international viewer. (Source: *Australasian Biotechnology*, vol. 9, No. 1, April 1999)

Public Opinion about Biotechnology: a Survey of Surveys

A 60-page, ring-bound publication providing a collation of public opinion surveys on biotechnology. It is intended as a reference guide to the public opinion survey information that is available in European and other countries. Produced by the Task Group on Public Perceptions of Biotechnology with the support of the European Commission DGXII. Available, Euro 11,—including postage from: Dr. Ana Maria Bravo-Angel, Assistant Coordinator Task Group on Public Perceptions of Biotechnology, European Federation of Biotechnology/Cambridge

Biomedical Consultants, Schuytstraat 12, NL-2517 XE The Hague. Tel. and Fax: +31 70 365 3857; e-mail: efb.cbc@ stm.tudelft.nl It is also downloadable from the following web site: http:/ www.kluyver.stm.tudelft.nl/ efb/tgppb/ home.htm

Embnet.news on Web

The latest issue of embnet.news, the newsletter of EMBnet, is available in html format on the web (www.ie. embnet.org/embnet.news) and in printable Postscript and Adobe Acrobat formats via ftp (ftp.ie.embnet.org/pub/ embnet.news). The newsletter contains information, articles, reviews, comments, and announcements of interest to the European and global bioinformatics communities. (Source: *Human Genome News*, 10(1-2) February 1999)

Unfinished microbial genomes searchable

The National Center for Biotechnology Information (NCBI) web site links to sequences from unfinished microbial genomes for BLAST searching (www.ncbi. nlm.nih.gov/www.BLAST/unfin_databases.html) These unfinished sequences, which are not yet in GenBank nor accessible via Entrez, also can be retrieved from their associated sequencing centres by ftp or web. The 18 finished microbial genomes are searchable by Entrez via the NCBI site (www.ncbi.nlm.nih.gov/Entrez/Genome/ org.html). Source: *Human Genome News*, 10(1-2), February 1999)

New HGMIS site translation of genetics to medicine

At the request of medical professionals eager for translation of genomics to medical practice, the Human Genome Project Information suite of web sites has added a new page called "Medicine and the New Genetics (www.oml.gov/hgmis/resource/medicine.html). This site covers topics of specific interest to physicians, nurses, genetic counsellors, and allied health professionals. It contains information and links about disease prevention, diagnosis and intervention; genetic-disease databases and support groups; gene testing; gene therapy; pharmacogenomics; genetic counselling; ethical, legal and social issues associated with genetics; continuing medical education courses in genetics; publications; multimedia; professional societies; and other resources. (Source: *Human Genome News*, 10(1-2) February 1999)

Genes on the Internet

The International Forum on Food and Agriculture, IFA, is a free-standing organization within the International Forum on Globalization (IFG), and addresses the global impacts of (industrial) agricultural production and distribution. Perhaps the best contribution of the site is its resource centre, which includes an electronic library. The library contains the full text of lots of current and relevant articles on agricultural issues, from biotechnology and agroecology to women in agriculture. The site is multilingual, supporting six languages. http://www.iffah.org/

The People and Plants Initiative—a joint project of WWF, UNESCO and the Royal Botanical Gardens, Kew—carries out applied research projects, community workshops, exchanges and training courses with young ethnobotanists from developing countries interested in conservation and community development. Its new web page aims not only to disseminate information, but also to create a space for discussion on the political issues raising from the commercial appropriation of ethnobotanical knowledge. http://www.rbgkew.org.uk/ peopleplants/index.html (Source: *Seedling*, March 1999)

Green chemistry network goes on-line

A database of Green Chemistry research projects in the UK will be put on the Internet by The Green Chemistry Network (GCN; York). According to GCN director James Clark, fellow of clean technology at The University of York, GCN will be linked to the Green Chemistry Institute, which provides information on research grants, chemical education, and conferences in the US.

The GCN web site will also offer information on funding opportunities, expertise, and examples of good environmental practice in chemical plants.

GCN will organize conferences, seminars and technology transfers. It also plans to make awards, beginning next year, to companies for case studies of green chemistry friendly production methods, as well as to scientists who have made significant discoveries.

GCN will also provide educational materials for schools and universities.

Although improving the public image of the chemical industry is not one of GCN's stated goals, Clark hopes that the network's activities will have that effect by making people more aware of the industry's efforts to make chemicals environmentally acceptable. The Royal Society of Chemistry (London) has provided GCN with a total of £500,000 ($850,000) for the next five years, and Clark is seeking additional funds from government agencies to support projects. (Source: *Chemical Week*, 13 January 1999)

Biocomputing

Biosafety WebPages

ICGEB invites you to visit its new "Biosafety WebPages" on Internet at the following address: http://www.icgeb.trieste.it/biosafety Your comments would be appreciated.

Electronic Journal of Biotechnology

The first issue of the Electronic Journal of Biotechnology is available on the Internet. In order to increase the visibility of the journal, there are two server locations: http://ejb.ucv.cl located in the southern hemisphere and http://ejb.org located in the US.

Companies are invited to publish advertisements free of charge in this journal. The classified section of this web site is http://ejb/feedback/classified.asp

Access to the FAO site on the Internet
http://www.fao.org

The FAO statistical database (http://apps.fao.org) complete sets of data on all relevant agricultural domains from around the world, covering 210 countries and territories and over 3,000 different items in the fields of agriculture, fisheries, forestry and nutrition.

FAO Events Calendar (http://www.fao.org/scripts/events) provides an interactive calendar of events, major meetings and conferences. The Conference, Council and committee meeting announcements provide a direct link to available meeting documents.

FAO Governing Bodies (http://www.fao.org/unfao/bodies) provides a description of the structure of the governing bodies and links to meetings documentation in Arabic, English, French and Spanish.

Employment Opportunities in FAO (http://www.fao.org/VA/Employ.htm). This is the site where all the FAO current vacancy announcements are posted.

WAICENT web page (http://www.fao.org/waicent). It is the entry page to the World Agricultural Information Centre (WAICENT), FAO's programme on information management.

FAO Documentation Catalogue (http://faowfs_h01.fao.org/library/ils_home1.html)—the complete catalogue of FAO documentation, on which searches can be done directly on the Internet.

FAO Publications Catalogue (http://www.fao.org/catalog)—up-to-date information on new publications and electronic products published by FAO.

Legal Office (http://www.fao.org/legal)—membership of the Organization, Basic Texts of the Organization, texts and status of treaties, and databases on national legislation on food and agriculture and coastal state requirements for foreign fishing.

Specialized information systems such as:

Global Information and Early Warning System on Food and Agriculture (GIEWS) (http://www.fao.org/waicent/faoinfo/economic/giews/english)

EMPRES Livestock Diseases Information System (http://www.fao.org/waicent/faoinfo/agricult/aga/agah/ empres)

World Information and Early Warning System on Plant Genetic Resources (WEIWS) (http://www.fao.org/waicent/faoinfo/agricult/agp/agps/pgr)

Domestic Animal Diversity Information System (DAD-IS) (http://www.fao.org/dad-is)

Information on the main technical areas of FAO can also be accessed at the following Internet addresses:

Agriculture (http://www.fao.orgwaicent/agricul/htm)

Nutrition (http://www.fao.org/waicent/faoinfo/economic/esn/nutri.htm)

Fisheries (http://www.fao.org/waicent/faoinfo/fishery/fishery.htm

Forestry (http://www.fao.org/waicent/faoinfo/forestry/forestry.htm

Sustainable development (http://www.fao.org/waicent.faoinfo.sustdev)

FAO News and Highlights (http://www.fao.org/news)—a central page regularly updated to provide information on major developments regarding food and agriculture

FAO In-depth Focus column (http://www.fao.org/focus/e/default.htm) provides textual and visual material to illustrate FAO's involvement in specific themes related to food security and sustainable agricultural development.

FAO Press Releases (http://www.fao.org/waicent/ois/press_ne/presseng/default.htm)

Visit Ag-West Biotech's Web Site

Ag-West's presence on the World Wide Web includes dozens of valuable features for the agbiotech community in Canada and around the world, including:

- Downloadable, full-format versions of The AgBiotech Bulletin and The AgBiotech Infosource;
- Dozens of informative articles provide current information on regulatory issues, intellectual property, economic development and the full range of issues covered in this newsletter;
- Dozens of connections will link you with economic development and funding agencies, biotech businesses in Saskatchewan and sources of biotech information around the world.

Visit the Ag-West web site at http://www.agwest.sk.ca

New York Biotech Association web site

The New York Biotechnology Association announced the launch of their new web site on 1 January 1999. www.nyba.org Featuring: Searchable Member Database, NYBA Events, Purchasing Consortium Information, Useful Links, Much, much more!

Elsevier web site

Elsevier Science announces the launch of the BIOtech Website, a gateway to important information about its scientific publications and databases in biotechnology and related disciplines, as well as access to a valuable bibliographic database of biotechnology articles—all provided at no charge. The BIOtech Website has been designed to keep investigators and information specialists in industry, academia and government informed about publishing initiatives in the multidisciplinary field of biotechnology throughout the Reed Elsevier organization.

Using a system of navigational links, visitors to the BIOtech Website are a mouse-click away from information about print publications (including journals, books

and magazines) and innovative electronic information services and data manipulation tools (web-based, traditional on-line files via major vendors, CD-ROMs and powerful graphical interfaces). Publications and services span fundamental research, industrial applications, patents, regulatory, legislative and business news in the following disciplines: agriculture and food; biochemical engineering and manufacturing; environment; life sciences; clinical and veterinary medicine.

Elsevier Science publishes peer-reviewed research articles devoted to biotechnology research, as well as the primary research fields that contribute to developments in biotechnology, in more than 235 journals and numerous books and reference works. The BIOtech Website also features a calendar of upcoming conferences and events, special discount offers and more.

Visit the BIOtech Website and bookmark for future reference: http://www.elsevier.nl/locate/biotech http://www.elsevier.com/locate/biotech (Source: *Australasian Biotechnology*, vol. 8, No. 6, December 1998)

A new biotechnology database from Elsevier Science

Elsevier Science, Secondary Publishing Division announces the launch of Biotechnobase, a unique bio technology database comprising more than 700,000 bibliographic citations and author abstracts from 1980 to the present. It provides comprehensive, international coverage of scientific, technological and professional biotechnology literature published in more than 280 journals (cover-to-cover)—from fundamental research to industrial applications. More than 90 per cent of records will include abstracts. The service is designed primarily for investigators, students, business developers and information specialists in industry, academia and government.

Biotechnobase will be available on-line via DIMDI beginning February 1999 (File: ET80). The file will be updated on a weekly basis. Mounting on other major on-line hosts will follow shortly thereafter. Further information: e-mail: embase-europe@elsevier.nl (Source: *Australasian Biotechnology*, vol. 9, No. 1, April 1999)