Leaking From The Lab?

The 'Contained' Use of Genetically Modified Micro-organisms in the UK

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1. SUMMARY

While there has been much concern about the safety of genetically modified crops and foods, releases of genetically modified micro-organisms (GMMs) are taking place, unmonitored, on a daily basis from factories and laboratories around the UK. This form of pollution is escaping control measures and could increase dramatically in scale if proposed new regulations are agreed.

GMMs are being used widely in the UK both for research purposes and by industry to produce enzymes, food additives and drugs. This is called 'contained use' to distinguish it from the deliberate release of other GM organisms such as GM crops. Although GMMs used in these ways are presented as being restricted to the laboratory or factory, they are in fact being incidentally or accidentally released in the workplace and into the environment. GMMs are required to be 'inactivated' before waste is discharged, but in the majority of cases this does not mean that all organisms must be killed. This report details GeneWatch's research into the use of GMMs, which has included reviewing the scientific literature; studying the public register of the use of GMMs in the UK; conducting a survey of large-scale users of GMMs; and making inquiries via officials and industry.

Bacteria, viruses, yeasts and fungi are all being genetically modified in the UK and there are 471 sites registered as using GMMs, mostly on a small scale for research purposes. However, this is an underestimate of the true figures because the Health and Safety Executive's (HSE's) public register was only introduced in 1992 and many facilities started using GMMs before that time.

Thirty-four centres (probably a large underestimate of the real number) are registered as using GMMs on a large scale, mainly for industrial use. However, there is no information available about what products are being developed from GMMs in factories. Drugs (such as insulin and antibiotics), enzymes and food additives could be, and probably are, all being made from GMMs in the UK. The use of GMMs could be on a huge scale. Although most waste is treated to kill the majority of the organisms before it is disposed of, some living GMMs are still released. Fermenters (in which organisms are grown in factories) can range from 10 to 10,000 litres in capacity containing up to 10^{14} or 10^{16} organisms in the larger fermenters (10^6 is one million organisms). Information on the public register shows that, after treatment to inactivate waste, companies still expect to be releasing waste containing hundreds, or even millions, of GMMs per litre. Extraordinarily, the Environment Agency, which is responsible for pollution control in the UK, has no information on where and how GMMs are being used in factories and therefore no knowledge of what GMMs are being released in waste streams or in aerial discharges by the companies involved. The HSE, which is responsible for implementing the regulations covering the use of GMMs, conducts no monitoring and no enforceable levels of allowed pollution are established.

GeneWatch has written to all the companies registered as using GMMs on a large scale. None of the companies using GMMs were prepared to supply details of what they were producing or releasing into the environment, their monitoring plans or data.

The main small-scale (less than ten litres) research uses of GMMs include the investigation of disease (especially cancer and infectious diseases) and the search for treatments in humans, animals and plants. Commercial research focuses on the use of GMMs to produce drugs and other products. The HSE estimates that there are around 5,500 new projects using GMMs on a small scale each year. There are no records of 90-95% of these because, once a laboratory is registered as using low-risk GMMs, there is no requirement to provide further information. The users conduct the risk assessments themselves and if they categorise a project as safe, no information is disclosed to the regulators. Only higher-risk GMMs which require tighter containment are scrutinised by the HSE.

Researchers at public institutes and universities appear to be the most irresponsible about the risks of GMMs even though they are often dealing with more dangerous organisms. The HSE has taken action against seven universities or institutes, including one (Edinburgh University) - twice, for failure to observe proper safety procedures:

November 1993:	National Institute of Medical Research - Improvement notices.
December 1993:	Birmingham University - Prohibition notice.
July 1994:	Kings College School of Medicine and Dentistry - Voluntary cessation of work. 3 improvement notices.
June 1995:	School of Hygiene and Tropical Medicine, London - Voluntary cessation of work. Improvement notice.
December 1996:	Institute for Animal Health, Pirbright - Improvement notice. Voluntary agreement that proposed work should not be undertaken until a full notification had been made.
July 1998:	University of Edinburgh - Improvement notice.
July 1998:	University College, London - Improvement notice.
February 1999:	University of Edinburgh - prosecuted and fined £3,500.

However, the failures identified so far are likely to be the tip of the iceberg since the HSE only has the equivalent of one person (in terms of hours allocated) dedicated to the inspection of the 500 sites using GMMs.

Although the use of GM techniques in research which is intended to bring human health benefits will probably be viewed much more sympathetically than the use of GM in crop and food production, risks to workers, the public or the environment should be avoided. The power of the HSE is restricted to determining the level of containment - not whether the GMMs should be produced at all. Experiments which may be considered irresponsible can be carried out and potential examples include the transfer of genes between two morbilliviruses - canine distemper virus and rinderpest virus. Morbilliviruses can cross species boundaries and, with very small changes, could cause dramatic alterations in their ability to cause disease.

Although many of the organisms involved in large-scale and research use are mainly classified as 'low risk', there is evidence that:

- even low-risk GMMs can survive for days or weeks in the environment;

- a GMM's foreign DNA can be passed to other organisms, and vice versa, with the potential to create new organisms which could alter ecosystems;
- so-called 'naked' DNA (DNA released from cells which have died and broken down) can be taken up by some bacteria;
- GMMs frequently contain antibiotic resistance genes, possibly increasing the likelihood of drug resistance appearing in disease-causing organisms;
- minor changes in genes can dramatically alter how dangerous an organism is;
- the vectors used to facilitate gene transfer in the laboratory may make gene transfer in the environment more likely.

The regulations covering the contained use of GMMs are about to be revised following the introduction of a revised Contained Use Directive in Europe. However, the new Directive weakens existing safeguards by removing the requirement to prevent the release of GMMs categorised as low-risk and by allowing for some GMMs to be exempt from any control. Because the revised Directive only sets *minimum standards*, the UK Government is free to impose stricter regulations to protect human health and the environment, but this opportunity has not been taken.

Instead, the Government proposes to remove the requirement to prevent the release of those GMMs categorised as low risk without any provision for independent monitoring, enforceable standards for containment, or a system to record all uses of GMMs. The user would be responsible for deciding whether a GMM was of low risk. Furthermore, the UK proposes introducing a mechanism to allow live GMMs to be released to the environment on a large scale without any treatment at all. In another proposal, 400-500 projects annually could be exempted from scrutiny. Disturbingly, rather than taking the opportunity to collect information, test scientific assumptions rigorously and learn more about GMMs, a naive faith has been placed in the ability of risk assessments to decide the likelihood and level of harm and, in the majority of cases, this decision is left to the GMM users themselves.

In the light of the research findings in this report, GeneWatch believes that the regulation of the contained use of GMMs must be brought into line with other pollution controls in the UK. To achieve this, and to improve the system more generally, GeneWatch recommends that:

More information must be obtained:

1. The HSE must backdate the public register to pre-1992 to include *all* centres registered as using GMMs. Information on the commercial use of GMMs must be collected and include data on the products manufactured from them. The proposed interim arrangements should be extended to include this.
2. Annual returns must be continued and extended to include lists of all risk assessments undertaken to enable scrutiny of the evaluations conducted by users of GMMs.
3. The public register must be made available via the Internet, should include a search engine and be comprehensive. Information must include details of the organisms involved, how they are modified, why the modification is

being undertaken, how the risk assessment has been arrived at, the dates use started and finished, what precautions are being taken to prevent release, and what monitoring takes place.

Risk evaluations must be improved:

4. In taking decisions about GMMs - and given the uncertainties involved and the potential for serious irreversible harm - a precautionary approach must be adopted.
5. Plasmids and naked DNA should be brought within the scope of the regulations.
6. Users must be required to present a worst case scenario when notifying the use of a GMM to reveal the full extent of the uncertainties.
7. The requirement for physical barriers to the release of GMMs should remain, together with the presumption (for all classes of GMMs) that there should be no releases of living GMMs into the environment. No discharges should be allowed unless reliable monitoring is available, a detailed risk assessment is presented which takes into account the local environment and the use of other GMMs, and a full justification for the need to discharge live GMMs or intact DNA is given.
8. Provisions for liability for any environmental harm arising from the use of GMMs should be included in the new regulations.

Pollution from GMMs must be monitored, policed and appropriate controls enforced:

9. The development of effective monitoring techniques must be a priority.
10. A legal system specifying the levels of GMM pollution that can be released in waste should be established. This would be consistent with other approaches to pollution control (e.g. chemicals), allow for prosecutions if breaches arise and drive a proper monitoring system.
11. The Environment Agency should be made responsible for independent monitoring of environmental releases of GMMs via waste streams and air and for the policing of discharges.
12. In addition, users of GMMs must be required to monitor to verify containment procedures and to implement systems for the detection of sudden leaks.
13. There must be increased investment in policing and enforcement.

Openness and transparency of the regulatory system must be established:

14. Refusal to disclose information about releases of GMMs to the environment on the grounds of commercial confidentiality must not be allowed under any circumstances. Users must supply details of any GMMs (including the species and how and why they have been genetically modified), the levels of release to the environment in waste and the monitoring systems in place.
15. Representation of public interest groups should be increased on the advisory committees, meetings should take place in public, and annual reports summarising each year's activities should be produced.
16. There should be greater public involvement in decision-making about the use of GMMs.

2. INTRODUCTION

The genetic engineering of crops and foods has become a controversial issue over recent years and public awareness is high. However, genetic engineering is also being used in other areas, some of which have received much less attention. One of these is the use of genetically modified micro-organisms (GMMs), such as bacteria, yeasts, fungi and viruses, both in public and private research laboratories and in commercial production facilities. This use is referred to as 'contained use' to distinguish it from other uses (in agricultural crop production, for example) where the genetically modified organism (GMO) is released deliberately into the environment.

Micro-organisms were the first organisms to be genetically engineered. In the early 1970s, key scientific developments allowed the function of individual genes to be identified; genes to be cut out from the genome using molecular 'scissors' called restriction enzymes; genes to be copied (cloned); and the transfer of 'foreign' DNA into bacteria, using vectors such as phages (infectious agents of bacteria) and mobile loops of bacterial DNA (plasmids) to transfer DNA. Together, these techniques form the basis of recombining genetic material from different species - so-called recombinant DNA technology or genetic engineering.

The scientists conducting the ground breaking experiments in the early 1970s were concerned about the potential for harmful impacts that might arise, such as the potential to create new pathogens. In 1975, the Asilomar conference in the USA and earlier deliberations of expert committees led scientists to introduce a voluntary moratorium on some laboratory experiments with genetically engineered micro-organisms until guidelines and regulations on their use were put in place. In the USA these took the form of guidelines, whereas in the UK voluntary controls were replaced by statutory regulations in 1978[1].

GMMs are being discharged into the environment either accidentally or incidentally

Since that time, the use of GMMs has become widespread both in university and industrial research laboratories and commercially to produce a wide array of enzymes (particularly for use in food processing and detergents) and drugs such as human insulin. GMMs are certainly being discharged into the environment either accidentally or incidentally through the breakdown of containment facilities or through routine discharges if the GMM is deemed 'safe'. Although the products of GMMs, such as drugs and enzymes for use in detergents, tend to be viewed with less hostility than some other products of genetic engineering, the impact of the living organism is of concern.

This report reviews the potential environmental and health risks of the escape of GMMs from both research and commercial facilities. The present regulations are described together with a description of GeneWatch's findings about how GMMs are being used and monitored in the UK. The European Directive intended to ensure the safe 'contained' use of GMMs (the Contained Use Directive, 90/219/EEC) has recently been revised and the UK has just (May 1999) published its plans to implement it. Therefore, this an important time to review the current status of GMMs in the UK, the risks involved and how these could be best avoided.

3. RISKS OF GENETICALLY MODIFIED MICRO-ORGANISMS

GMMs could cause harm in several ways. Firstly, if they are pathogenic (able to cause disease) in humans or animals, they could cause illness in the people working with them or more widely if they escape from the laboratory. Secondly, they could survive in the environment and disrupt natural microbial ecosystems. If they continued to produce a certain product (such as an enzyme or antibiotic), they could be directly damaging to organisms. Thirdly, the foreign DNA could move into other species, altering them in unpredictable ways. Because DNA from dead cells can be taken up into living cells, even so-called 'naked' DNA (DNA which is not contained in a cell) has the potential to have effects.

Table 1 summarises the questions that are thought relevant to the assessment of the effects of releases of GMMs into the environment. Three of the most important questions in determining the environmental effects of a release of a GMM are its characteristics, whether it is likely to survive outside the laboratory or factory environment, and whether foreign genetic material can be transferred to other organisms. If an organism can survive and/or transfer genetic material, questions arise about the implications of this.

Escaped GMMs could cause illness, disrupt natural microbial ecosystems and alter other species in unpredictable ways

Table 1: Data requirements to predict the effect of the release of a GMM to the environment (adapted from Doyle *et al* (1995)[2])

QUESTION	FACTORS AFFECTING OUTCOME
Survival of GMM – establishment and multiplication.	Nature of organism, such as its ability to cause disease.
	Effect and nature of the genetic modification – does it give a competitive advantage?
	Scale and frequency of release.
	Receiving environment – including biological and physical characteristics.
Dispersal of GMM to other sites.	Characteristics of environment.
Transfer of foreign genetic material to other organisms.	Survival, establishment and multiplication of organisms.
Ecological impacts of GMM and foreign DNA.	Interactions with other organisms and effect of product(s) of GMM.
Potential for containment, decontamination and mitigation if adverse effects detected.	Nature of receiving environment and scale of effects.

3.1 Survival of GMMs in the Environment

A great range of organisms have been genetically modified, including viruses, bacteria and yeasts. Some of this work involves organisms able to cause disease

in humans, animals or plants. Other work uses organisms which, in their natural state, are not harmful.

In theory, many of the GMMs used in contained facilities have either been bred in laboratories over many generations and lost their ability to survive in the natural environment or have had specific sequences inserted or deleted to reduce their ability to survive. For example, the *Bacillus* organisms used by the Danish enzyme company, Novo Nordisk, to produce protease and amylase have had genes removed making them asporogenous, so only one cell in 10 million is able to form a spore. Spore forming ability, when an organism develops a protective coat and can survive longer in the environment, is an important characteristic of the organism.

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Eschericia coli (*E.coli*) K12 is another of the most commonly used bacteria in research and is a disabled strain which it is assumed cannot survive outside the laboratory. The *E.coli* K12 strain has probably been engineered and manipulated by human beings more than any other strain of bacteria. It was originally isolated in 1922 from the faeces of a diphtheria patient at Stanford Medical School and has been maintained under laboratory conditions since then. Other commonly used species include the bacteria *Bacillus* sp*; Streptomyces* sp*; Kluyveromyces* sp; *Trichoderma* sp*; Klebsiella* sp; the yeast, *Saccharomyces cerevisiae* and the fungus, *Apsergillus niger.*

Particular attention has been given to the ability of *E.coli* K12 to survive and colonise because some strains of *E.coli* can be pathogenic and cause intestinal disease. There are a great number of K12 derivatives with various mutations which should make them unlikely to survive or compete well. However, there is evidence that these disabled organisms can survive outside the laboratory, although the length of survival depends on a variety of factors related to the organism and the environment. In the intestines of experimental animals, various strains of *E.coli* K12 which had been genetically engineered to produce bovine somatotrophin (BST) or human growth hormone (HGH) and were resistant to one or more antibiotics survived for up to 7-14 days but did not appear to colonise the intestine even in the presence of selective pressure in the form of the relevant antibiotic[3,4,5,6]. Similarly, various other strains of *E.coli* K12 survived for around 4-6 days in the human intestine but did not colonise longer term[7,8].

Although *E.coli* is an organism which is normally found in the intestines of animals, it can survive in the wider environment. The Health and Safety Executive (HSE) guidelines on risk assessment state that *E.coli* K12 can survive for 7 days in external environments[9]. However, other research indicates this may be an underestimate in some circumstances although there is great variation between studies, probably related to differing experimental conditions. For example, Tschäpe[10] showed that *E.coli* K12 could survive in a small sludge unit - although the *E.coli* could not be detected for 12 days, it eventually 'reappeared' having acquired an additional plasmid which appeared to confer no competitive advantage. Other research has shown that a genetically engineered *E.coli* K12 strain survived for at least 35 days in a non-sterile silt loam soil[11]. In contrast, in other studies, a BST strain of *E.coli* K12 was eliminated from sewage sludge over 5-6 days following a single, high dose innoculum[12].

….yet there is evidence that 'disabled' organisms can survive outside the laboratory

E.coli K12 can also survive in river and sea water for periods of well over 2 months if the water is sterile but only for periods of about 2-18 days if the water is untreated[6,13]. This is thought to be due to competition with other organisms in non-sterile conditions. Survival times are much longer at lower temperatures.

There is less published information on the survivability of many of the other strains used in genetic modification experiments although some organisms used in laboratory work are quite robust. For example, *Pseudomonas putida* UWC1 survived for 8 weeks in a sewage activated sludge unit[14] although other strains may have shorter or longer survival periods.

GMMs may not only survive in water, soil or air, they may also be ingested by invertebrates which could affect their survival and their distribution. This is an issue which has only recently been addressed and experiments have shown that a genetically modified *Pseudomonas fluorescens* can survive and multiply in the intestines of the earthworm, *Octolasion cyaneum*[15], and the woodlouse, *Porcellino scaber*[16]. Because these organisms are consumed by others, GMMs and DNA could move through the food web.

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Laboratory techniques may not be able to identify all living organisms in the environment so those experiments which have been done may underestimate survival rates. Some organisms enter what is referred to as a 'viable, non-culturable' (VNC) condition[17,18.] That is, although an organism may not grow on the culture media used in laboratories, it may still be alive and able to multiply in the correct environmental conditions. This possibility was identified because there are often differences between visual counts of bacteria (based on their ability to take up certain stains, which is thought to indicate metabolic activity) and numbers isolated by culture. Numbers cultured tend to be lower than those considered viable by staining techniques, leading to the hypothesis that bacteria may enter a dormant phase which conceals their viability when cultured on artificial media. This has been challenged on the grounds that staining may not provide an accurate indication of viability[19], but the large amount of literature demonstrating VNC for such a large number of species suggests it is not a spurious observation.

Knowledge of disease transmission has shown that viruses can survive in air and be transported over long distances, a characteristic which is very important in the spread of some viral diseases such as foot and mouth disease. Whether a virus can survive in air depends on its own characteristics, such as coat lipid content, and the physical conditions of the air such as humidity. Viruses are also spread in the environment via faeces or other discharges from infected animals. However, because viruses are much more difficult to isolate than bacteria (see Section 6.3.2) there is much less information about their persistence in the environment.

It is clear, therefore, that even disabled organisms have the potential to survive for many days or weeks in the environment. Because of the VNC condition, it may be difficult to determine survival rates of micro-organisms with confidence. In addition, the potential exists for GMMs to move through the food web if they are ingested by organisms which may, in some cases, improve their likelihood of survival.

For organisms which are known to cause disease, even though they may not survive for long periods, they could still cause harm in the short term if they escape confinement and encounter a susceptible person, animal or plant.

3.2 The Transfer of Genetic Material

Even if GMMs do not become established in the environment in the long term, it is possible that they could either pass their foreign genetic material to other organisms or else acquire the ability to become established from others. This movement of genetic material between organisms is known as 'horizontal transfer' to differentiate it from the vertical transfer between one generation and the next. Over the past twenty years, there has been a burgeoning literature about gene transfer between micro-organisms leaving the impression - reinforced by the way in which antibiotic resistance has spread between bacterial species - that it is an extremely important and influential process.

There are three mechanisms by which horizontal gene transfer is thought to take place:

Transformation: The uptake of free ('naked') DNA from the environment and its incorporation into the bacterial genome.

Conjugation: Movement of DNA between bacteria following cell-to-cell contact and effected by plasmids or transposons.

Transduction: The transfer of genetic material from one bacterium to another by a bacteriophage (an infective virus of bacteria).

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3.2.1 Transformation

The process of 'natural genetic transformation' is restricted to bacteria and involves the uptake of naked DNA (of chromosmal or plasmid origin). For transformation to take place, there must be free DNA in the environment which can be taken up by bacteria and bacteria must be able to take up DNA – a state which is known as 'competence'[20]. It has been known for a number of years that extracellular DNA exists in the environment, most of which is of microbial origin. This DNA is released when cells die and start to degrade, but can also be excreted at other times such as during cell growth and during spore germination.

Competence, when bacteria can bind extracellular DNA and take it in, is not present in all species of bacteria and even in those species which show competence it may vary according to environmental conditions. For example, in *Neisseria gonorrhoea* competence is persistent, in *Haemopillus influenzae* it can be induced under conditions which inhibit growth, and in *E.coli* competence is difficult to induce often requiring laboratory techniques such as electroporation[21].

DNA is broken down at high rates when initially introduced into waste water, seawater, freshwater sediments and soils[20]. There is evidence that this degradation is caused by a mixture of micro-organisms producing DNase (an enzyme that degrades DNA)[22]. Despite the high level of DNases found in a whole range of environmental samples, extracellular DNA has been found consistently in

a variety of habitats. This is partly because DNA is produced continually by micro-organisms, but also because in some circumstances DNA can avoid being degraded.

Extracellular DNA has been associated with cellular slime which it is believed may stabilise the DNA structure – up to 40% of the dry matter of cellular slime can be DNA[20]. DNA may also be protected through its ability to form complexes with various minerals such as clay, feldspar, heavy metals, and humic substances. Adsorption of DNA to sand or clay particles is thought to protect DNA against DNase activity and, although adsorption slows the process of transformation, uptake of adsorbed DNA does take place and does not require a desorbtion step before it can take place[23]. There are a variety of factors which affect the rate and extent of this protective adsorption of DNA by minerals. For example, the type of mineral and its acidity or alkalinity (pH) both have a large effect, although binding can occur over a wide range of pH values. The shape and size of the DNA molecule and general temperature have a much lesser effect[20].

The inability of an organism to survive does not mean that its genetic material could not be transferred to other species

DNA may persist for considerable periods of time. For example, using PCR analysis, which can identify very small quantities of specific DNA, it was found that DNA from a genetically engineered *E.coli* K12 remained undegraded for at least 40 days in a silt loam soil[24].

The evidence from microcosm and other studies that transformation can take place both in aquatic and terrestrial environments involving both chromosomal and plasmid DNA[25,26,27] suggests that transformation may be a significant route of gene transfer between bacterial species. The frequencies of such transformation events may be low, making detection difficult, but the findings show that the inability of an organism to survive does not mean that its genetic material could not be transferred to other species.

3.2.2 Conjugation

Plasmids are the most widely used tool to introduce new DNA sequences artificially into micro-organisms…

Conjugation is the most studied form of gene transfer between bacteria. It involves DNA exchange following cell-to-cell contact and is mediated by some (but not all) plasmids and transposons. Plasmids are circular strands of extra chromosomal DNA and transposons are mobile genetic elements which are capable of integration into both chromosomal or plasmid DNA. Both plasmids and transposons can carry, and are thought to be responsible for, the widespread occurrence of antibiotic resistance genes and both are used in genetic modification techniques. Plasmids are the most widely used tool to introduce new DNA sequences artificially into micro-organisms, but in order for a plasmid to transfer genes between bacteria under natural conditions they require certain characteristics:

- the ability to produce pili (thread-like structures which bind the two cells together) and enzymes necessary for replication and transport of DNA;
- a sequence of DNA called *Tra* which will allow conjugative plasmids to move between one cell and another (to be transmissible);

In addition, plasmids have specific host ranges which may be narrow or broad[26] so they may be able to transfer DNA between one or two species or across a whole range of unrelated species.

One of the most important safety mechanisms in the production of GMMs is the use of plasmids which are deficient in one or all of these transfer mechanisms and have a restricted host range. However, although such precautions will reduce the risk of transfer, it is possible for the plasmids in such a GMM to acquire the ability to undergo conjugation. For example, *E.coli* cells containing a non-conjugative recombinant plasmid have been shown to be capable of receiving a conjugative plasmid from another *E.coli* strain[28]. If the recombinant plasmid contained the *Mob* sequence, it was then capable of transferring itself into a third *E.coli* strain by utilising the structures and enzymatic properties of the conjugative plasmid. Similarly, non-conjugative plasmids in *P. putida* in activated sludge units acquired the ability to conjugate in the presence of other bacteria. Bacteria isolated from waste water were able to mobilise a recombinant non-conjugative plasmid from *E.coli* K12[29]. About 50% of *E.coli* strains from human volunteers were able to promote the transfer of a recombinant non-conjugative plasmid from *E.coli* K12[30]. However, the rate of this transfer was low and the resulting organisms did not colonise the intestinal tract of mice.

…"there is no such thing as a safe plasmid"

In addition, it has been shown that some conjugative transposons can also transfer into plasmids and facilitate mobilisation[31] which has led to the observation that *"there is no such thing as a safe plasmid"* [32].

Recent research has shown that gene transfer between the laboratory strains, *E.coli* K12 and *E.coli* B can take place in the digestive vacuoles of a protozoan, *Tetrahymena pyriformis*[33]. Such free-living protozoa are widespread in the environment, would normally ingest many released GMMs and many survive the digestive process. If an innocuous GMM was ingested at the same time as a pathogen or an organism that contains a plasmid that could restore conjugative properties to the plasmid of the GMM, the *E.coli* could acquire such genes.

Earthworms have been shown to increase the distribution through the soil of both a genetically modified *P. fluorescens* and soil organisms which acquired the plasmid it was carrying by conjugation[34].

There is considerable evidence, not least from the spread of antibiotic resistance, that conjugation is an extremely important mechanism for sharing genetic material between bacteria in natural systems. Complete confidence cannot be placed in the steps taken to limit gene transfer by conjugation.

3.2.3 Transduction

Transduction, mediated by phages, may only be important for the exchange of genetic material between closely related species, because phages have a limited host range[21].

Phages are infective agents (viruses) of bacteria which are able to pick up, carry and inject DNA into a new host. The DNA may then be integrated into the host genome or into a plasmid where it may persist. A phage could infect a GMM and transfer the foreign DNA to another organism.

Although there is evidence that a large number of phages exist in the environment, there are few data about the frequency of transduction in the wild and thus its significance for GMMs is difficult to assess.

3.3 The Effect of the Inserted DNA

Although safety mechanisms may be built into GMMs, they are by no means foolproof. The exact nature of the inserted foreign DNA will influence the impact any GMM has if it escapes confinement and particular areas of concern include:

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- **The use of antibiotic resistance marker genes.** This is very common practice as a way of identifying when a genetic modification has been successful. The release of GMMs with antibiotic resistance genes could exacerbate the present problems with antibiotic resistance in disease-causing organisms if they spread to other organisms. It is argued that such genes are ubiquitous in nature but the scale, sites and nature of any releases have the potential to increase the risk.
- Gene transfers which could alter the host range an organism can infect or, if transferred to other organisms in the environment, could increase their pathogenicity. A single gene transferred from *Yersinia pseudotuberculosis* to *E.coli* K12 enabled it to invade mammalian cells in culture[35]. Conceivably, so-called 'pathogenicity islands', which are regions of DNA that contain a variety of virulence genes[36], could be transferred.
- The introduction of genes from vectors (the plasmids, transposons and phages used in genetic modification) which facilitate the transfer of DNA. There are mechanisms which act as obstacles to the transfer of foreign DNA. For example, restriction enzymes can recognise and cut up such DNA so that it is not incorporated. However, by using genes and gene sequences which can overcome these defences, there are fears that gene transfer could increase in frequency and make a harmful effect more likely to occur[37].

3.4 Evaluating the Impacts of GMMs in the Environment

The effects of any obviously pathogenic GMMs which are released into the environment are relatively easy to assess. However, the majority of organisms which are used are not overtly pathogenic and, although the GMM may persist in the environment and transfer foreign genetic material, predicting the impact of this is difficult. This is largely because so little is known about the ecology of micro-organisms in the environment. Nevertheless, the natural microbial flora are unlikely to be unimportant either in ecosystem or human health terms and their disturbance by GMMs could be significant. Issues which need to be addressed include:

- **The impact of vector systems developed to facilitate gene transfer in the laboratory.** The presence of GMMs in the environment containing gene sequences from these systems may pose special risks by increasing the likelihood of gene transfer through overcoming natural barriers.
- **The impact of antibiotic resistance genes used as markers in GMMs.** The presence of increased levels of antibiotic resistance genes could make the treatment of bacterial diseases more difficult if they were to be transferred to disease-causing organisms.
- **The impact that the products of some GMMs (such as enzymes and drugs) may have on the environment.** If a GMM which was designed to produce a drug or enzyme survives in the environment, it may continue to produce the product. This chemical may have effects on other bacteria or other components of the ecosystem.

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Until our basic knowledge of microbial systems improves, ignorance dominates any risk assessment.

4. THE REGULATORY FRAMEWORK

The existence of risks associated both with the use of GMMs and their escape to the environment has long been recognised and has led to the evolution of a set of regulatory controls which are intended to prevent harm occurring (see Box 1). The UK regulations currently in place implement the European Union's Contained Use Directive (90/219/EEC), but an amended version of this Directive has recently been agreed and UK regulations will be changed as a result, the consultation process beginning in May 1999. Both the Contained Use Directive and the revised Directive set baseline levels of protection and Member States are able to introduce more stringent regulations.

Being able to place a GMM in the appropriate risk group and containment class is fundamental to the success of the safety system

Although the Contained Use Directive only includes GMMs in its scope, the UK regulations also cover the contained use of GM plants (e.g. in greenhouses) and GM animals. However, this report only considers GMMs.

The way in which the regulations currently operate is shown in Box 2. Based upon a risk assessment process, the GMM is placed in either a low or higher risk category and, for the higher risk group, given an appropriate containment level. The actual assessment is undertaken by the person, institution or company wishing to undertake the work, with policing and granting of approvals the responsibility of the HSE. Being able to place a GMM in the appropriate risk group and containment class is fundamental to the success of the safety system.

4.1 Risk Assessment

The UK's approach to risk assessment is based upon a determination of whether the GMM poses a risk to human health or the environment, together with the scale of its use - large (usually industrial) or small (usually research). Depending on the risk category into which an organism is placed, the assessment may or may not be subjected to independent scrutiny.

All users of GMMs have a legal responsibility to "limit contact with the general population and the environment"

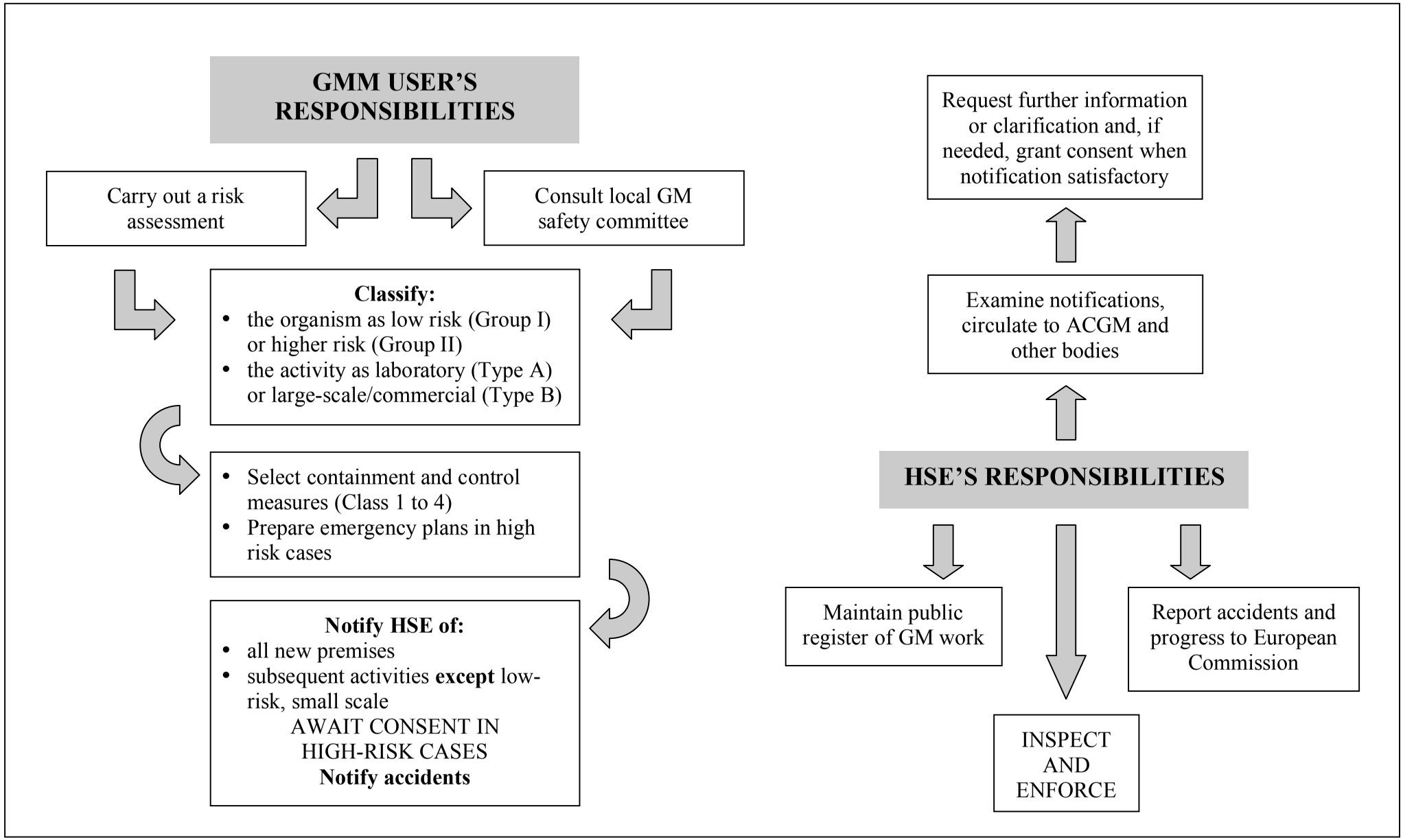
4.1.1 The Approach to Risk

The definition of contained use in the Contained Use Directive does not mean that organisms which are regulated by it should be kept in absolute containment. All users of GMMs covered by the Directive have a legal responsibility to *"limit contact with the general population and the environment"*. This is undertaken through a combination of physical, biological and chemical containment measures. Physical containment includes measures such as air filtration systems, protective clothing, and the ability to fumigate and isolate premises as mechanisms to prevent a GMM physically escaping. Biological containment involves changes to the organism which mean that if it does escape it cannot survive, cause disease or other harm. These systems include disabling organisms so they cannot form spores, for example. Alternatively, they may be deficient in a replication factor which can only be supplied in the laboratory, or have plasmids which lack the ability to be mobilised. Chemical containment includes the use of disinfectants to clean work surfaces, fumigation of laboratories and chemical 'kill tanks' where chemicals are used to kill organisms which have been used in production systems.

Box 1: The History of the Regulation of the Laboratory Use of Genetically Modified Micro-organisms in the UK [1,38,39]

1972	First successful transfer of DNA between different species.
1972	UK Government sets up working party under Lord Ashby which recommends strict safeguards be established.
1975	Asilomar conference in California – scientists agree to a voluntary moratorium on some recombinant DNA experiments until guidelines or regulations are in place.
1976	UK working party recommends establishment of a Genetic Manipulation Advisory Group (GMAG) to examine proposals for GMO work.
1978	Regulations introduced to make notification of laboratory use of GMOs to GMAG compulsory under Health and Safety at Work Act 1974.
1984	GMAG becomes the Health and Safety Commission's Advisory Committee on Genetic Manipulation (ACGM).
1989	Regulations extended to include the release of GMOs to the environment.
1990	The term '*manipulation*' in the ACGM's title changed to '*modification*'.
1993	New regulations introduced under Health and Safety at Work Act 1974 to implement the 1990 European Directive on the Contained Use of GMOs – *Genetically Modified Organisms (Contained Use) Regulations 1992 (as amended by the Genetically Modified Organisms (Contained Use) Regulations 1996)*. These cover the laboratory and greenhouse use of genetically modified micro-organisms, plants and animals.
1993	Separate regulations introduced to cover the release of GMOs to the environment.
1996	ACGM establishes new technical sub-committee (TSC) to advise on technical questions.
1998	Following industry pressure to relax regulatory control of the contained use of GMOs, a new European Directive (98/81/EC amending Directive 90/219/EEC on the contained use of genetically modified micro-organisms) is introduced.
1999	Consultations on the revision of UK regulations in the light of the new European Directive.
2000	5th June implementation deadline for revised regulations.

Box 2: How the Contained Use Regulations Operate



The actual containment level is decided upon by comparing the properties of the GMM with those of the parental strain and with other organisms that have been classified already. This method of risk assessment relies upon being able to predict the properties of the GMM by assuming the risk will be equal to the sum of the GMM's constituent parts (the parental strain, the inserted gene sequence and the vector).

GMMs are assigned to one of four categories depending on the risk group of the organism (I or II) and the scale of production (Type A or B). The four categories are:

IA low risk, small scale (usually research) - e.g. the use of *E.coli* K12 in a university laboratory.

IB low risk, large scale (usually for industrial production) - e.g. the use of *E.coli* K12 in a fermenter (over 10 litres in capacity) to produce the drug bovine somatotrophin (BST).

IIA higher risk, small scale - e.g. the use of a potential pathogen such as influenza virus in a university or company laboratory.

IIB higher risk, large scale - e.g. the use of a potential pathogen to produce a drug (N.B. there are none of these in the UK at present).

Risk assessments are undertaken by the GMM user in consultation with a local GM safety committee – there is no formal scrutiny of the categorisation by the HSE

In the case of Group I GMMs, there is only a legal requirement to follow 'good microbiological practice'. However, where Group II status has been allocated, it is also necessary to determine a specific containment level for the GMM. There are four containment levels with 1 being the most lax and 4 being the most stringent, and categorisation is based on an assessment of their potential to cause harmful effects to human health or the environment. These containment levels are defined in Annex IV of the Contained Use Directive. It is only at containment levels 3 and 4 that the requirement to *prevent* rather than *limit* release exists. The main features of increasing containment are more physical barriers to escape since the classification of a GMM in the higher risk category acknowledges that it would cause harm if it escaped.

Before using GMMs, the person or company intending to do so must register the centre where the work is to be carried out with the HSE. The HSE evaluates the proposal and the centre's suitability and decides whether or not to grant its approval. If the centre is approved for 'low risk' Group I organisms (either Type A or B), different GMMs may be produced subsequently without further approval as long as they fall within the Group I category. For centres approved for Group II use, each new use must be notified to and, in the case of Group IIB organisms, approved by the HSE. The risk assessments are undertaken by the GMM user in consultation with a local GM safety committee – there is no formal scrutiny of this categorisation by the HSE.

The UK guidelines outline how organisms should be categorised and recommend safety measures to mitigate against some risks such as gene transfer by stating that *"plasmid vectors should be immobilised"* (Schedule 2 Paragraph 5) and this is more strictly interpreted for large scale operations. Thus, for Type A operations, organisms should be Tra^-, Mob^+ (not transmissible but mobilisable) and for Type B operations, GMMs should be Tra^- Mob^- (neither transmissible nor mobilisable).

4.1.2 Assessing the Human Health Risks

Risk assessment for human health is usually based upon the 'Brenner Scheme'. This works by applying numerical values to the following characteristics of the GMM:

Access – the likelihood that the organism could enter and survive in a human.

Expression – a measure of the level of expression of the cloned protein.

Damage – the potential for the organism or the expressed protein to cause harm.

These values are combined to give an indication of the appropriate containment level. However, as the HSE acknowledges, the Brenner Scheme may not always give a reliable indication of risk. This is because there are circumstances where there may be effects which cannot be predicted from experience with other (non-GM) organisms which is the basis of the Brenner Scheme. For example:

Introduced genes may alter or exacerbate existing pathogenic traits unpredictably

- introduced genes (e.g. pathogenicity determinants or antibiotic resistance genes) may alter or exacerbate existing pathogenic traits unpredictably;
- when there is uncertainty over the level of attenuation (the weakening of an organism to make it less able to cause disease or survive) of the host strains;
- when completely new types of constructs (e.g. deletion mutations) are formed from a plasmid vector and an inserted coding sequence.

The shortcomings of simply comparing GMMs to non-GM organisms is seen in the case of GM baculoviruses. Baculoviruses may be used in genetic modification as vectors to transfer genes into other micro-organisms. It had been assumed that baculoviruses were not capable of infecting human or plant cells and so were not hazardous to workers. However, recent studies have shown that baculoviruses can infect mammalian cells and, when combined with mammalian promoters, can result in the expression of foreign genes in those cells[40]. If baculovirus vectors were to infect the cells of workers, they could be exposed to the products (such as drugs), which could prove harmful, and this has necessitated a reassessment of the risk assessment procedure for baculoviruses[41].

4.1.3 Assessing the Environmental Risks

Environmental harm is not precisely defined in the regulations but is related to effects on overall populations and ecosystems rather than harm to an individual, as may be the case in dealing with endangered wild animals. In contrast to the health risk assessment, there is no attempt to quantify the environmental risks.

The procedure for assessing environmental risk is:

- hazard identification;
- assessment of the likelihood of any identified hazards being manifested;
- assessment of the consequence of the identified hazards being manifested;

- determination of risk of 'harm' (likelihood multiplied by consequence);
- management (control) of risk.

Although there is a requirement for those using GMMs to carry out an environmental risk assessment, these are extremely poorly conducted in practice. The criticisms one of the HSE's advisory committees, the ACGM Technical Sub-Committee, has made about certain environmental risk assessments include:

"A number of statements were made, e.g. that the overall risk to the environment was effectively zero, without any supporting evidence. The assessment of risk was in several instances based upon assertion rather than evidence." [42]

"... the notifications generally dealt very poorly with these [environmental] aspects. There was, in particular, an absence of justification for statements that the work posed no risk to the environment." [43]

"Dr Bowden commented that a lot of centres did not appear to be aware of what was required in an environmental risk assessment." [44]

Although there is a requirement for those using GMMs to carry out an environmental risk assessment, these are extremely poorly conducted in practice

4.1.4 Uncertainty in Risk Assessments

Genetic modification allows organisms to be altered in very dramatic ways. Many of the functions of micro-organisms - such as why they do or do not cause disease - are complex and poorly understood. In both the human and environmental risk assessments there are clearly considerable areas of uncertainty and ignorance.

This risk assessment procedure relies fundamentally on the parental strain having been correctly assigned a containment level. As more and more vectors are constructed and GMMs are themselves further modified, any mistakes which are made may become compounded.

As well as this uncertainty surrounding the classification of risk, other issues are neglected. Neither when assessing risk to human health nor the environment is the effect of naked DNA taken into account. Each organism is considered in isolation and other operations taking place at the site are not considered. If the same centre is used for several different GMMs, unexpected recombinations may arise leading to previously innocuous organisms becoming harmful.

"The assessment of risk was in several instances based upon assertion rather than evidence."

4.2 Advisory Committees

The HSE has two main advisory committees involved in GMMs – the Advisory Committee on Genetic Modification (ACGM) and its Technical Sub-Committee (TSC). The ACGM deals with matters of policy and the TSC advises on scientific and technical matters.

The current members of the ACGM and the TSC are listed in Appendix 1. The HSE does not hold any information about the financial or other interests of members which might influence their opinions about the risks of GMMs. The committees are made up largely of academics and CBI and TUC nominees who

may also be academics. One industry employee is on the ACGM and chairs the TSC. The HSE say they are to add a second CBI nominee and an independent 'public interest' representative to the ACGM 'soon'[45]. There is no public interest representative on the TSC although there is a CBI and a TUC nominee.

The HSE does not hold any information about the financial or other interests of advisory committee members which might influence their opinions about the risks of GMMs

Neither the ACGM or its TSC prepare an annual report, making it difficult to gain an overall picture about the committees, how they operate and make their decisions. However, one area where the TSC excels in relation to other committees working in the area of GMOs - such as the Advisory Committee on Releases to the Environment (ACRE) and Advisory Committee on Novel Foods and Processes (ACNFP) - is in the publication of detailed minutes (since early 1998) of their meetings which have comments attributed to individual members. This provides useful insights into the recent deliberations behind decisions and often demonstrates that there is considerable questioning of some proposals. It also provides the public with an opportunity to question further from a basis of knowledge. However, the removal of some sections on grounds of confidentiality makes some issues frustratingly difficult to understand.

4.3 Regulatory Monitoring Requirements

The EU Contained Use Directive refers to monitoring of GMM facilities to determine if organisms have escaped *"when necessary"*. This is implemented in the UK regulations as Article 12 (1) (d) of the Genetically Modified Organisms (Contained Use) Regulations 1992 which requires users of GMMs:

> *"(d) to test, when necessary, for the presence of viable organisms outside the primary physical containment"*.

The HSE's Guidelines to the Regulations give an indication of when they consider monitoring necessary. There is none specified for small scale work at any containment level. For large scale low risk GMMs, the HSE's advice is that:

> *"Monitoring is unlikely to be required for many activities at Level B1. However, where there is a risk to human health or environmental safety from process organisms outside the closed system, monitoring for viable process organisms should be carried out."*

There is no requirement in the Regulations for the HSE to undertake routine, independent monitoring and the HSE has not undertaken any

For higher risk, large scale use, the HSE advises:

> *"Where there is risk to human health or environmental safety from process organisms outside the closed system monitoring for process organisms should be carried out."*

There is no requirement in the Regulations for the HSE to undertake routine, independent monitoring and the HSE has not undertaken any. The information that is available suggests that monitoring varies from being limited to non-existent (see Section 6.2 below).

4.4 Enforcement

One of the roles of the HSE is to inspect premises where GMMs are used. For the approximately 500 sites registered as using GMMs, 1,665 person hours (225 days) are allocated annually for inspections of GMM facilities[46]. This is equivalent to one person working full time.

Following inspections, the HSE has the power to issue either improvement or prohibition notices to centres or to prosecute them if they do not comply with the regulations. Improvement notices instruct the user to take specified actions such as rectifying shortcomings in risk assessments, and prohibition notices prevent further work on a GMM project until specified safety measures have been introduced and this has been verified by the HSE. Since 1992, five improvement notices have been served, one prohibition notice served (University of Birmingham), and there has been one prosecution (University of Edinburgh) (see Table 2).

DATE	GM CENTRE	BREACH OF LEGISLATION	ENFORCEMENT ACTION
Nov 1993	National Institute of Medical Research	Failure to undertake a written assessment for a containment level 3 project	Improvement notices
Dec 1993	Birmingham University	Inadequate risk assessment and the use of containment facilities that failed to meet the requirements of containment level 2	Prohibition notice
July 1994	Kings College School of Medicine and Dentistry	Shortcomings in work procedures and facilities used for work at containment level 3	Voluntary cessation of work. 3 improvement notices
June 1995	School of Hygiene and Tropical Medicine, London	Inadequate risk assessments, failure to notify Group II work and shortcomings in work procedures and facilities used for work at containment level 3	Voluntary cessation of work. Improvement notice
Dec 1996	Institute for Animal Health, Pirbright	Inadequate risk assessments, failure to notify a number of Group II projects	Improvement notice. Voluntary agreement that proposed work should not be undertaken until a full notification had been made
July 1998	University of Edinburgh	Failure to undertake risk assessments or hold GM safety committee meetings	Improvement notice
July 1998	University College, London	Failure to notify a containment level 3 project concerning HIV virus	Improvement notice
February 1999	University of Edinburgh	Failure to respond to improvement notice and carry out risk assessments	Prosecuted and fined £3,500

Table 2: Enforcement action taken by HSE on centres not complying with the Contained Use regulations

The improvement and prohibition notices suggest that research workers take a particularly cavalier approach to the risks associated with the use of GMMs. Risk assessments have not been undertaken, containment has not been adequate and the HSE has not been notified about projects. These were not trivial failings. For example, University College, London did not notify the HSE about its work with genetically modified human immunodeficiency virus (HIV). The University of Edinburgh did not appear to consider the risks sufficient to heed an improvement notice served on them and were successfully prosecuted, although the £3,500 fine is hardly punitive for such a large institution.

Improvement and prohibition notices suggest that research workers take a particularly cavalier approach to the risks associated with the use of GMMs

Given the tiny commitment which the HSE makes to inspection, it is likely that the breaches they identify are the tip of the iceberg. Coupled with a lack of monitoring, those institutions failing to comply with regulations are unlikely to be detected.

4.5 Public Information

The regulations allow for certain information to be made available about the use of GMMs in a public register held by the HSE. Additional information about centres can be requested by application to the HSE and this includes annual returns which users are expected to complete and details of risk assessments carried out. However, information may be withheld on the grounds of commercial confidentiality.

4.5.1 The Public Register

Under the Contained Use Directive there is provision for the supply of public information. In the UK, this takes the form of a public register, held and administered by the HSE, but this only began in 1992 after the introduction of the Contained Use Directive. For information about GMMs registered before that time, application has to be made to the HSE under the freedom of information provisions. However, this approach depends on knowing what information exists and what questions to ask.

The centre or person intending to use GMMs (the notifier) is asked to give the following information:

- name and address of organisation (and department if applicable);
- the purpose of the genetic modification;
- a description of the genetically modified organism(s) involved or intended to be involved;
- the methods for monitoring the genetically modified organisms and for emergency responses (if any);
- an evaluation of foreseeable effects and, in particular, any pathogenic and ecologically disruptive effects created by the genetically modified organisms involved.

In reality, the public register consists mainly of undated, single sheets of paper which outline the work being proposed when each centre first gave notification and is a cursory summary of the assessment required by the HSE. The quality of the information on these sheets varies enormously. Some give one sentence answers while others include attached sheets which go into much more detail. Because the register was first set up in 1992 and many laboratories and industrial facilities had already registered as Group I, A or B users, they are not included in the register.

4.5.2 Commercial Confidentiality

Companies can withhold information on the grounds of commercial confidentiality although they must justify their reasons for doing so. However, they also exploit loopholes in the regulations to withhold information. GeneWatch requested more information about the large scale commercial activities (Group IB) and commercial research use of Group II organisms registered with the HSE. However, Delta Biotechnology, SmithKline Beecham and Zeneca all withdrew some or all of their notifications. They are allowed to do this if they no longer undertake or never started the work originally notified. This means there could be no publicly available historical information on past (now completed) uses of GMMs and possible releases to the environment. GeneWatch had to obtain this information under the Freedom of Environmental Information Regulations.

Information about the inactivation of waste referred to in Zeneca BioProducts' notification of the large scale use of GMMs to produce human lactoferrin was denied on the grounds of commercial confidentiality

Some information about waste treatments is also withheld because of commercial confidentiality. For instance, GeneWatch requested more details about the inactivation of waste referred to in Zeneca BioProducts' notification of the large scale use of GMMs to produce human lactoferrin at Billingham in Cleveland, but this was denied on the grounds of commercial confidentiality. The information requested was data referred to in the application showing the degree to which the GMM was killed by heat treatment before discharge into waste. It is difficult to understand why this is of commercial significance and seems to be a misuse of the exemption to deny the public access to information of environmental importance which would reveal the extent of discharges of GMMs.

4.5.3 Annual Returns

Notified centres are expected to supply an annual return to the HSE about their activities. GeneWatch has examined several of these and found that they provide very little useful information. Only the *number* of risk assessments undertaken are given, not what they involve. Gathering such data would assist inspectors in detecting when GMMs may be wrongly classified as low risk.

4.5.4 Accidents and Emergencies

Notified centres are also expected to inform the HSE of any accidental releases or accidents that arise in the use of GMMs. Rather implausibly, in the seven years since the regulations were first introduced, it seems there has never been a single

accident[47]. This is probably because an accident only has to be reported under UK regulations if its release would be considered harmful. Since users of Group I GMMs normally claim that their organisms would be harmless if released to the environment, they escape recording. By contrast, in Denmark, Novo Nordisk reported accidental releases of GMMs from one of their enzyme factories in 1997 and they have occurred at other times[48].

The public register, and information which can be unearthed from it, is hugely inadequate and has been given low priority

4.5.5 Shortcomings in Public Information

It is clear that the public register and information which can be unearthed from it is hugely inadequate and has been given low priority. For example, there is not even a date on the summary sheets included in the public register. As described below, the inadequacies of the public register (particularly the lack of information before 1992; lack of recording of the risk assessments undertaken by notified centres; and lack of information about when activities started and ended) means that it is impossible to gain an accurate picture of the use of GMMs in the UK.

5. THE USE OF GENETICALLY MODIFIED MICRO-ORGANISMS IN THE UK

In an attempt to discover the scope of use of GMMs in the UK, GeneWatch consulted the HSE's public register on Contained Use, made further inquiries through the HSE, sent a letter to all those installations registered as using GMMs on a large scale and made a search of relevant Parliamentary Answers. Because this research mainly elicited data on where GMMs were being used rather than what they were being used for, other research was conducted using trade associations and official documents.

There are probably around 500 sites using GMMs in the UK including centres notified before 1992 and not included on the public register. This number includes both Group I and II organisms being used on a small and large scale. No absolute figure is available because prior to the introduction of the Contained Use regulations, such data was not in the public domain. Since 1992, there have been 275 centres registered for Group I work (34 of which have notified their intention of working on a large scale), and 196 for Group II work[49]. The HSE has estimated that about 5,500 new projects with GMMs are undertaken each year, 90-95% of which are classified as Group IA[50].

There are probably around 500 sites using GMMs in the UK including centres notified before 1992 and not included on the public register

5.1 Small-Scale Use of GMMs

The largest number of small-scale research centres are using Group I GMMs on a small scale. There are probably around 300 such sites in the UK.

When GeneWatch examined the HSE's public register, there were a total of 191 Group II centres which fall into three major categories as follows:

Universities	- 92 centres in 38 universities*
Research Institutes	- 57 centres in 45 research institutes
Companies	- 42 centres in 36 companies

*This treats University of London colleges as separate universities.

It is impossible to systematically analyse what research work is undertaken because:

- Centres using Group I organisms only have to register once and do not supply any information about subsequent research.
- Many centres were registered before 1992 and so no public information exists.
- The HSE has no easily usable data – there is no public computer database for example and no search facilities to make analysis feasible.
- Notifications can be withdrawn on grounds of commercial confidentiality if the work has finished or did not take place.
- There are no dates on the public register so it is impossible to know when sites were registered.
- The information on the public register is often very vague. For example, Glaxo Research and Development Ltd in Stevenage state the purpose of their genetic modification to be '*research*' using '*various*' GMMs. It is

possible to obtain further information about a particular centre on application to the HSE but some of the information will remain confidential.

The use of GMMs on a small scale is mainly for medical and other scientific research. In industrial laboratories, the production of chemicals/drugs is another important research dimension which includes:

- GMM pharmaceutical manufacturing processes (e.g. SmithKline Beecham Pharmaceuticals, Worthing; British Bio-technology, Oxford; Chiroscience, Cambridge; Genzyme, West Malling);
- vaccine production (e.g. Medeva Group Research, Speke);
- diagnostics (e.g.Amersham International, Cardiff).

Because it is not possible to undertake a comprehensive survey of the scientific research uses, the following gives an example of the sorts of work being undertaken and highlights some areas where there may be particular concern over work with human and domestic animal disease and pathogens, and plant viruses and other pathogens.

5.1.1 Human and Domestic Animal Disease and Pathogens

Inevitably, the vast majority of research with GMMs - both in commercial and public facilities - is being undertaken into diseases in humans. A smaller amount of work concerns disease in domestic animals. The work being undertaken is very varied but includes:

- the genetic manipulation of disease-causing micro-organisms to understand better how they cause illness or to develop vaccines;
- the genetic manipulation of human or animal cells to understand disease processes, susceptibility and resistance to disease.

There are serious risks from certain genetic modifications, both for the workers involved and the wider public or animal life should the GMM escape containment

Understanding cancer and developing treatments is a particularly common area of research and there is undoubtedly a great deal of public sympathy for this kind of work. However, there are serious risks from certain genetic modifications, both for the workers involved and the wider public or animal life should the GMM escape containment.

Many experiments are being planned which have the potential to alter the spectrum of species in which a micro-organism may be pathogenic. For example, the Institute of Animal Health at Pirbright, is transferring genes from canine distemper virus (CDV) into rinderpest virus (RPV) and investigating the effect on the ability of the resulting virus to infect laboratory species[51]. Neither of these morbilliviruses infect humans, but others (such as measles virus) do. Morbilliviruses have shown themselves able to cross species boundaries and a great deal of uncertainty exists about how they cause disease. Animal tests have limited predictive ability and may be misleading. For example, Sendai virus (another morbillivirus) was lethal in one strain of mice supplied in the UK, but not in the same strain of mice supplied from Japan[52]. Very small changes in a cell may also have a dramatic effect. A 1,000 fold increase in Senadi virus pathogenicity was seen when a single protein was modified[52]. If the pathogenicity

of such GM viruses was increased and they escaped, they could cause disease in workers and the public.

These kinds of risks are only evaluated according to what level of containment is required - whether they need to be conducted in level 3 or 4 facilities, for example. Although there were questions from members of the technical sub-committee of the ACGM about the scientific justification for the CDV/RPV experiments, they have still been approved, in part because *'it [is] not HSE policy to decide whether work should proceed, but whether it [is] sufficiently contained'* [53]. This means that an assumption has to be made that the level of containment will be 100% effective in preventing any harm arising. However, if this does not prove to be the case, it will be very difficult, if not impossible, to justify the consequences.

Another area of concern is where genetic modification experiments are being undertaken to investigate and manipulate pathogenesis mechanisms. The National Institute for Medical Research (NIMR) at Mill Hill, asked the HSE for permission to genetically modify human influenza virus with a gene from the avian influenza virus. Although this work is not thought to be proceeding, the research could have resulted in the production of a flu virus very similar to that which caused the influenza epidemic of 1918, killing many tens of thousands of people. The risk assessment conducted by the NIMR in the event of the GM influenza virus escaping was said by an HSE reviewer to '*..indicate a lack of understanding of the potential risk and an attitude which, in my opinion, greatly increases the risk'* [54].

One risk assessment was said by an HSE reviewer to '..indicate a lack of understanding of the potential risk and an attitude which, in my opinion, greatly increases the risk'

The investigation of how cancer arises may put workers at risk if cancer-causing genes (oncogenes), tumour viruses or mutagens are being used. In the case of cancer-causing genes, there is evidence that 'naked' (no longer contained in a cell) oncogenes can cause tumours in laboratory animals and there have been reports of an increased incidence of cancers in scientists working with such genes[55].

GMMs are also being used in defensive biological warfare research at the Defence Establishment Research Agency (DERA - previously the Chemical and Biological Weapons Defence Establishment) at Porton Down, using a range of human and animal pathogens such as *Salmonella typhimurium, Clostridium perfringens* and *Yesinia pestis* (the bubonic plague organism).

5.1.2 Plant Viruses and Other Pathogens

A smaller amount of research is being undertaken into plant viruses and other pathogens. The dangers of using GM plant viruses are similar to those associated with human and animal pathogens. If there are unexpected changes in the spectrum of plants a virus can infect or its pathogenicity, both crops and wild plants may suffer if they escape confinement. Despite the Contained Use regulations having been introduced in 1992, the HSE still does not have guidelines covering the use of GM plants in containment (which will be used in experiments with plant pathogens). Furthermore, the Ministry of Agriculture, Fisheries and Food (MAFF), which is responsible for non-GM plant pathogen work, has not been aware, in at least one case, of the requirement for centres using

GM plant pathogens to inform the HSE and undertake the appropriate risk assessment[56].

There is very little information about the exact nature of the plant pathogen work which is taking place and there appears to be less discussion of it at the ACGM or its Technical Sub-Committee whose preoccupation seems to be with risks to human health. Centres registered to use GM plant pathogens include the John Innes Centre at Norwich, the Institute of Virology at Oxford and the Scottish Crop Research Institute.

There are 34 centres registered as using Group I GMMs on a large scale. This is likely to be a significant underestimate of the true figure

Other pathogen work at Zeneca's Jealott's Hill Research Station includes the large scale use of a GM yeast including gene sequences from an insect virus. This is being developed to make the yeast infectious to certain insects for use in biological control techniques.

5.2 Large-Scale Use of GMMs

According to data supplied by the HSE and given in reply to a Parliamentary Question, there are 34 centres registered as using Group I GMMs on a large scale (Group IA - see Appendix 2). Because other centres were registered before 1992 and are not included on the public register, this is likely to be a significant underestimate of the real number of sites where GMMs are used on a large scale. According to the HSE, no Group II organisms are being used on a large scale.

However, a GeneWatch survey of the companies and institutes has shown that the HSE's list of large scale users of GMMs is out of date. For example, one institution (Plymouth Marine Laboratory) was wrongly listed as undertaking Group IB work in information supplied to GeneWatch. Because there is no requirement to inform the HSE when projects start or finish, several others replied that although they may have once been registered as undertaking Group IB work, they were no longer involved in such activities.

Companies are generally unwilling to reveal any information about their activities (see Appendix 2). Furthermore, there are no public data held on what substances are being produced by companies using GMMs on a large scale in the UK because *'where the substance produced is not a live GMO this falls outside the scope of the Contained Use Regulations'* [57]. Not even the Environment Agency has such data even though it is responsible for effluents from industrial facilities[58].

"For discharges to water …there is no requirement to inform [the Enivronment Agency] if GM material (whether de-activated or not) is present."

The kinds of products which may be produced by GMMs include enzymes, food additives, and human and veterinary medical products. Currently, only GM bacteria and yeasts are used in commercial production systems.

5.2.1 Enzymes

Enzymes produced by GMMs are used in food processing (see Table 3), in detergents or other industrial processes. The GMMs are grown and multiplied in a fermenter in a factory and the product is extracted from the resulting mix. As far as GeneWatch can determine, only one enzyme from a GMM - pullulanase - is produced in the UK at Rhodia Enzymes, a subsidiary of Rhône Poulenc. The others are imported, mainly from other parts of Europe.

Table 3: Commercially Available Enzymes Made By Genetically Modified Micro-organisms For Use In Food Processing
(source: Association of Manufacturers of Fermentation Enzyme Products)

ENZYME	HOST ORGANISM	DONOR ORGANISM	USE	MAIN APPLIC-ATIONS
Alpha-acetolactate decarboxylase	*Bacillus amyloliquefaciens* or *subtilis*	*Bacillus* sp	Brewing - reducing maturation time	Bevr
Alpha-amylase	*Bacillus amyloliquefaciens* or *subtilis*	*Bacillus* sp	To degrade starch. Used in baking and brewing to make more sugars available for yeast fermentation. Used in detergents to break down starch in food stains.	Stch, Bevrs
	Bacillus lichenformis	*Bacillus* sp		Stch, Frut, Bevr, Sugr, Bake
Catalase	*Aspergillus niger*	*Aspergillus* sp		Milk, Egg
Chymosin	*Aspergillus niger* var. *awamori*	Calf stomach	To 'clot' milk and separate curd from whey in cheese making	Cheese
	Kluyveromyces lactis	Calf stomach		Cheese
Cyclodextrin-glucosyl transferase	*Bacillus lichenformis*	*Thermoanbacter* sp		Stch
Beta-glucanase	*Bacillus amyloliquefaciens* or *subtilis*	*Bacillus* sp	Glucan degredation, beer filtration, fruity aroma in wine	Stch, Bevr
	Tricoderma reesei or *longibrachiatum*	*Trichoderma* sp		Stch, Diet
Glucose isomerase	*Streptomyces lividans*	*Actioplanes* sp	To make fructose syrup	Stch
	Streptomyces rubiginosus	*Streptomyces* sp		Stch
Glucose oxidase	*Aspergillus niger*	*Aspergillus* sp	Formation of gluconic acid; food preservation.	Egg, Bevr, Bake, Sald
Hemicellulase	*Bacillus amyloliquefaciens* or *subtilis*	*Bacillus* sp	To alter gluten in wheat Used in bread making to improve texture and colour.	Bake
Lipase, triacylglycerol	*Aspergillus niger*	*Candida* sp *Rhizomucor* sp *Humicola* sp	To break down fats in baking industry and in the production of fats and oils.	Fats Fats Fats, Bake
Maltogenic amylase	*Bacillus amyloliquefaciens* or *subtilis*	*Bacillus* sp	Starch modification	Stch, Bevr, Bake
Protease	*Aspergillus oryzae*	*Rhizomucor* sp	To break down proteins	Cheese
	Bacillus amyloliquefaciens or *subtilis*	*Bacillus* sp		Meat, Fish, Stch, Bevr, Bake
	Bacillus lichenformis	*Bacillus* sp		Meat, Fish
Pullulanase	*Bacillus lichenformis*	*Bacillus* sp	Debranching of starch	Stch
	Klebsiella planticola	*Klebsiella* sp		Stch, Bavr, Bake
Xylanase	*Aspergillus niger* var. *awamori*	*Aspergillus* sp	Degradation of gluten in flour.	Bake
	Aspergillus niger	*Aspergillus* sp		Stch, Bevr, Bake
	Bacillus amyloliquefaciens or *subtilis*	*Bacillus* sp		Stch, Bevr, Bake
	Bacillus lichenformis	*Bacillus* sp		Stch
	Tricoderma reesei or *longibrachiatum*	*Trichoderma* sp		Stch, Bevr, Bake

These enzymes are used to treat ingredients for use in a processed food product

Key: Bake = Bakery; Bevr = Beverages (soft drinks, beer, wine); Cheese = cheese; Diet = Dietary food; Egg = egg; Fats = fats & oils; Fish = fish; Meat = meat; Sald = salads; Stch = cereal and starch; Sugr = sugar and honey.

5.2.2 Food Additives

There are also several food additives which can be produced by GMMs in the same way as enzymes. No comprehensive list is available but they include riboflavin and aspartamate[59].

An increasing number of drugs and vaccines are being produced using GMMs

5.2.3 Human and Veterinary Drugs and Vaccines

An increasing number of drugs and vaccines are being produced using GMMs. A non-exhaustive list is given in Table 4.Antibiotics are not included, although it is likely that these are being produced using GMMs in some cases. Many of these will not be produced in the UK, but it is equally likely that some are. Pharmaceutical companies notified as centres using GMMs on a large scale were approached for this information but refused to provide it on grounds of commercial confidentiality.

Table 4: Medical products made using GMMs

DRUG	TRADE NAME	COMPANY	APPLICATION
Alteplase	**Activase**	Genentech	Heart disease
Interferon beta 1	**Avonex**	Biogen	Multiple sclerosis
Factor IX	**BeneFix**	Genetics Institute	Haemophilia B
Interferon beta 1-B	**Betaseron**	Berlex Laboratories/ Chiron	Multiple sclerosis
Factor VIII	**Bioclate Helixate**	Centeon	Haemophilia A
Alglucerase	**Cerezyme**	Genzyme	Type 1 Gaucher's disease
Follitropin beta	**Follistim**	Organon Inc.	Infertility
Insulin	**Humalog**	Eli Lilly	Diabetes
Insulin	**Humulin**	Eli Lilly	Diabetes
Interferon alphacon-1	**Infergen**	Amgen	Hepatitis C
Factor VIII	**Kogenate**	Bayer	Haemophilia A
CSF/Leukine Liquid	**Leukine**	Immunex	Bone marrow transplantation and leukaemia
Insulin	**Novolin**	Novo Nordisk	Diabetes
Somatrophin	**Nutropin/ Nutropin AQ**	Genentech	Growth hormone deficiency
Somatrem	**Protropin**	Genentech	Growth hormone deficiency
Alpha dornase	**Pulmozyme**	Genentech	Cystic fibrosis
Factor XIII	**Recombinate**	Baxter Healthcare (Genetics Institute)	Haemophilia A
Hepatitis B vaccine	**Recombivax-HB**	Merck	Hepatitis B vaccine
Reteplase plasminogen activator	**RetavaseTM**	Centocor, Inc.	Heart disease
Interferon alpha - 2a	**Roferon-A**	Hoffmann-La Roche Inc.	Leukaemia, AIDS-related Kaposi sarcoma and hepatitis C.
Growth hormone	**Saizen**	Serono Laboratories Inc.	Growth hormone deficiency.

The medicines and vaccines included on this list are produced and/or developed by companies involved in recombinant DNA research or other biotechnology applications.

Source: The Biotechnology Industry Organization, http://www.bio.org/bioproducts/guide99.html

6. GMMS IN THE ENVIRONMENT

There is no explicit legal requirement to kill all GMMs to be disposed of in waste if they have been deemed 'safe' and are considered to have a limited ability to survive in the environment (Group I organisms). The HSE have said that, in their experience, large scale facilities routinely inactivate all GMMs prior to disposal. However, according to officials in the HSE, when waste is said to be inactivated, this does not mean that all organisms must be killed although the majority should be. The waste from research facilities may be heat treated in autoclaves or chemically treated before disposal by methods such as incineration, but there is no independent verification that this is undertaken. Therefore, Group I organisms will be entering the environment in waste from both industrial and research facilities using these organisms.

In their applications to the HSE to register uses of GMMs, companies have acknowledged that releases will take place even if cultures are claimed to have been inactivated:

- In their 1993 notification of large scale use, Zeneca BioProducts at Billingham expected releases of 10^4-10^6 organisms per millilitre of a GM *E.coli* K12 producing the enzyme, xylanase. According to their environmental risk assessment, the GMMs were expected to be released into the following sites: *"Terrestrial, research and production site. Water drainage/sewage system"*.
- In another 1993 notification of large scale use, Zeneca BioProducts at Billingham expected treatments to reduce levels in waste to around 100 organisms per millilitre of a GM yeast, *S. cerevisae*, which produces human serum albumin.
- In a 1994 notification of large scale use, SmithKline Beecham Pharmaceuticals in Irvine, Ayrshire, acknowledged that GM *Penicillium chrysogenum* was *'rarely'* released via air and effluent. Air sampling is conducted weekly.
- In various notifications, SmithKline Beecham Pharmaceuticals at Worthing, West Sussex, claim the likelihood of release of GMMs used at the site will be *'low'* as they are killed prior to disposal by a method they decline to disclose because of commercial confidentiality.

According to officials in the HSE, when waste is said to be inactivated, this does not mean that all organisms will be killed

In commercial situations, the economic value of GMMs gives an incentive to limit leakage through the process plant. GMMs are grown in large vessels called 'fermenters' and the end product extracted from them. Following this, the remaining GMM waste is treated in a kill tank to inactivate organisms before discharge. Quality control, especially in pharmaceutical production, will help limit escape at all stages and so will the desire to protect the intellectual property of the company – they do not want competitors to have access to their organisms. This has led to an improvement in the containment practices compared to the production of traditional fermentation products, such as brewing or vinegar production, which are often carried out in large open vessels[60]. However, leaks, especially in the form of aerosols (droplets in the air) but also as fluids, can occur as a result of:

- structural damage or failure of a seal in the fermentation vessel or pipe work – aerosol leaks;

- overpressure in fermentation tank leading to large scale release caused by failure of a safety device – aerosol leaks;
- leakage during inoculation, addition to or sampling of the fermentation tank – aerosol leaks;
- handling of the product after fermentation – aerosol leaks;
- leakage during processing post fermentation, e.g. during centrifuging or filtration – aerosol leaks;
- effluent disposal following 'kill tank' treatment – fluid discharge.

Fermenters are sealed and pressure tested, normally operate at comparatively low pressures and are fitted with bursting discs designed to fail if the vessel is significantly over pressure. Fermenters rarely fail but it can happen[61]. Kill tanks, where organisms are 'inactivated' before release to waste by either heat or chemical treatment are never 100% effective in killing organisms and if this system fails it could lead to huge numbers of live organisms being released in effluent.

The numbers of organisms involved in a release, either in an aerosol or fluid discharge, will not be trivial in the industrial setting

The numbers of organisms involved in a release, either in an aerosol or fluid discharge, will not be trivial in the industrial setting. Fermenters can range in size from 10 to 10,000 litres in capacity with up to 10^{14} or 10^{16} organisms in larger fermenters. Even the release of 1% or 0.1% will involve many millions of organisms.

In Denmark, which is home to one of the largest enzyme producers using GMMs, Novo Nordisk, rather than obscuring the fact that the release of GMMs takes place, a different approach is taken. The Danish regulations accept that, when used on a large scale, some GMMs can be released so companies are given release limits which they must monitor and report[48].

6.1 Monitoring for Releases

At the present time there is no independent monitoring of containment and releases of GMMs by the HSE or any other authority. Any monitoring that takes place is undertaken by the user and the results are not available to the public.

In 1996, the HSE sent a questionnaire to 49 companies using GMMs[60]. The companies were asked what sampling methods they used to monitor process organisms inside and outside the workplace. Responses were received from 33 companies (see Table 5). Less than half (14 out of 33) of those who responded carried out regular monitoring. All seven in production scale use monitored, most but not all (9 out of 11) pilot scale plants carried out monitoring, but less than half (10 out of 28) of the laboratory scale operations included any monitoring at all. These smaller scale users tended to rely on 'good microbiological practice' to ensure absolute containment. All but one of the companies that did not monitor were small scale operations in university or research council laboratories.

At the present time, there is no independent monitoring of containment and releases of GMMs by the HSE or any other authority

The majority of the monitoring took place inside the workplace with only five companies monitoring outside as well as inside the workplace.

Table 5: HSE Questionnaire Results (from Crook and Cottam (1996)) [60]

QUESTION	GMO	NON GMO	TOTAL
Nature of the process organism used at the time the questionnaire was administered	24	9	33
Companies at which regular workplace monitoring was taking place	12	2	14
Companies at which monitoring was being done both within and outside the workplace	4	1	5

There are no standard methods for monitoring micro-organisms and no guidelines from the HSE as to appropriate methods to use.

GeneWatch wrote to all those facilities listed as using GMMs on a large scale to ask about their monitoring techniques and frequencies. None of them supplied any details of their monitoring.

6.2 Where Monitoring is Necessary and the Difficulties Involved

Designing and developing systems which are reliable and effective is not easy but is essential if the assumptions behind risk assessments are to be tested and compliance with containment is to be determined.

6.2.1 Where to Monitor

Monitoring must be comprehensive if it is to detect leaks and monitor routine discharges. The sites where detection methods are needed include:

The aim should be to obtain results very rapidly because if there is a leak, it needs to be identified and stopped immediately

1. Within the factory or research laboratory for the early detection of leaks in equipment, thus preventing damage to employees' health, ensuring no economic loss and minimising the risk of environmental release.
2. At factory or laboratory outlets to monitor continually, if possible, the numbers of GMMs released into the environment. If monitoring here is accurate, it can provide a measure of how many live organisms are released into the environment. This is particularly necessary at air and gas release outlets, because once organisms have moved into the wider atmospheric environment they will be massively diluted.
3. External environmental monitoring:
    a) to detect any surviving GMMs,
    b) to detect horizontal gene transfer,
    c) to look for any effects the released organisms might have.

In principle, the aim should be to obtain results very rapidly because if there is a leak it needs to be identified and stopped immediately.

6.2.2 Difficulties in Monitoring

The main method used to detect GMMs is by culture. However, viruses are especially difficult to identify as they require cell culture techniques for their isolation. These are both time consuming (weeks not days) and technically demanding. In reality, there are no practicable ways of monitoring for GM viruses yet developed. It may only be through evidence of their impact (such as an outbreak of human, animal or plant disease) that they will be detected.

For bacteria, the situation is somewhat easier. Culture techniques are routine and take 2-3 days, but because many of the cells have been disabled, traditional cultivation techniques are not applicable. In addition, when exposed to environmental stress, many bacteria may remain viable but become non-culturable (VNC) and cannot be counted on agar plates. These methods also do not detect 'naked' DNA. Furthermore, effluent leaving a plant or laboratory will contain a mixture of killed and living organisms and DNA, making monitoring for live cells difficult.

As well as issues related to the organism itself, the environment in which the organism is found can also cause difficulties. Monitoring in the environment is more difficult than in the process plant or laboratory, and air, water and soil each have their own specific problems. Monitoring of air requires incredibly sensitive techniques due to the massive dilution that takes place. However, compared with other media, air contains a relatively small variety of micro-organisms. In water, organisms can be hugely diluted and difficult to identify. In soil, there is less chance of dilution, but the number of other micro-organisms is massive (approximately 10^9 g^{-1}) and the ecology of soils very complex.

It may only be through evidence of their impact (such as an outbreak of human, animal or plant disease) that GM viruses will be detected

6.2.3 Methodologies

Despite the difficulties involved in monitoring, these are not insurmountable. There are various methods which can be used in different circumstances. None are ideal for every situation and a combination will be needed. In Denmark, Novo Nordisk carry out their external monitoring by the use of selective media, and then colonies with the same phenotype are isolated and PCR used to identify the modified strain.

Marker genes – these are inserted into the GMM in order to give it an easily distinguishable feature. Antibiotic resistance is not useful in this context because many soil micro-organisms often have resistance to at least one antibiotic[62]. Other approaches include the incorporation of genes which code for bioluminescent or fluorescent molecules which can then be measured, or the inclusion of genes for enzymes which catalyse a colour change. There are drawbacks to these approaches. For example, the production of luminescence can be reduced by environmental stress and, in aquatic environments, the presence of indigenous luminescent bacteria will distort counts.

Selective media – because microorganisms have different nutritional and environmental requirements for growth, special media can be used to limit the range of micro-organisms grown on it. It can be sensitive and simple but the organism must be viable and culturable. Results can take up to 4 days which

means this method is not appropriate for continuous monitoring or to check for accidental releases from a process plant. Other similar organisms will also grow and this becomes a problem in identifying micro-organisms which are present in low quantities.

There is clearly an urgent need to develop methods which are effective, rapid and accurate in identifying GMMs….

DNA probes – perhaps the most specific method of detecting both GMMs and naked DNA is through the use of DNA probes. These 'probes' recognise and bind to the foreign DNA inserted in the organism, are very specific and, if combined with techniques such as PCR, can detect low levels. Because they do not indicate if an organism is viable they may need to be combined with other methods.

Immunological techniques – using antibody detection systems and radio-immuno assay. These do not detect whether organisms are living but are sensitive to specific changes in the GMM and so can assist in their identification against a background of other organisms.

Sampling – different sampling techniques are needed according to the medium and organisms to be isolated and the identification method used. A full review is beyond the scope of this report, but techniques include sampling on plates, collection for other forms of detection and more general methods such as aerosol detection systems to alert for leaks inside the workplace.

…Until such tests are developed, it is difficult to understand how releases of GMMs can be justified

There is clearly an urgent need to develop methods which are effective, rapid and accurate in identifying GMMs. Military interest in biological weapons is leading to systems which can rapidly identify biological agents in air. The task for users of GMMs should be much easier as they know which organisms to look for, and knowledge derived from military research could advance civilian detection systems enormously. Until now, there has been little incentive to develop monitoring methods as there are no legally specified requirements. Demands for the detection of GM ingredients in food over the past six months have led to the rapid emergence of accurate and sensitive tests. If such pressures were brought to bear over the need to detect GMMs, tests are likely to emerge just as rapidly. Until such tests are developed, it is difficult to understand how releases of GMMs can be justified.

7. REVISION OF THE EU DIRECTIVE 90/219/EEC

In December 1995, following heavy industry campaigning, the European Commission decided to revise the 1990 Contained Use Directive. Industry had argued that the Directive needed to be streamlined and authorisation made easier, claiming that the safety requirements put European companies at a competitive disadvantage compared to their main competitors.

A revised Directive has now been agreed (98/81/EC amending Directive 90/219/EEC on the contained use of genetically modified organisms) and has to be implemented by Member States by 5th June 2000. Because the Directive sets out *minimum* standards, Member States may introduce tighter regulations. UK regulations will have to be amended and the HSE has just begun a consultation process on how this should happen.

Because the Directive sets out minimum standards, Member States may introduce tighter regulations

7.1 The Revised Directive

The main changes to the Directive and their implications are described below.

7.1.1 Scope of the Directive

The new EU Directive continues to cover only GM micro-organisms (including animals and plant cells in culture). Naked DNA and plasmids are still not included.

7.1.2 The Definition of Contained Use

Currently, the definition of contained use stipulates that '*physical barriers, or a combination of physical together with chemical and or biological barriers*' should be used to *'limit their* [GMMs] *contact with the general population and the environment'*. The new Directive defines contained use as:

> *"...any activity in which micro-organisms are genetically modified ...and for which specific containment measures are used to limit their contact with the general population and the environment"* (Article 2 (c)).

By removing the requirement for physical barriers, with or without chemical and biological control measures, biological barriers alone - such as the inhibition of sporolation, the use of non-mobilisable plasmids and disabled strains such as *E.coli* K12 - are sufficient to satisfy the definition of 'contained'.

By removing the requirement for physical barriers, biological barriers alone are sufficient to satisfy the definition of 'contained'

This change constitutes a weakening of the present system as it could allow the discharge of GMMs into the environment which previously would have had some form of physical containment and inactivation before discharge.

7.1.3 Classification System

Under the present system, GMMs are classified into two groups depending on the perceived risk of the organism and on two levels depending on the scale of the

operation. In the revised Directive, this is replaced by a system based on four classes of containment regardless of the scale. Class 1 organisms will be deemed to carry the least risk and Class 4 the highest risk.

The specifications for containment are much more precise. There are now specific criteria for each type of work - for instance, in laboratories, in glasshouses and growth rooms, in animal units and *'for other activities'* (really commercial use). There is now a distinction between waste treatment for *'effluent from hand washing-sinks and showers or similar'* and *'GMMs in contaminated material and waste including those in process effluent before final discharge'*. There is no requirement for waste from Class 1 organisms to be inactivated before discharge. This is optional and dependent on the risk assessment.

7.1.4 Exclusions

The possibility of excluding certain GMMs from the Contained Use regulations is probably the biggest change to the Directive. Yet the criteria for deciding which will be excluded will not be announced until the legislation is due to be implemented.

In an effort to speed up the whole process for users of GMMs, the revised Directive is reducing the period of time that is allowed to the competent authority to assess applications to use GMMs

7.1.5 Notification Procedures

In an effort to speed up the whole process for users of GMMs, the revised Directive is reducing the period of time that is allowed to the competent authority to assess applications to use GMMs. Generally, the notification periods have now been decreased to include, in some instances, immediate starts (Class 1, all but first time users). For other Classes, the time limit for the HSE to decide whether or not to authorise is reduced from 90 to 45 days. This could result in less rigorous scrutiny of proposals as advisory committees may not meet frequently enough to consider them.

7.1.6 Information Available to the Public

In both the new Directive and in current legislation, it is up to the notifier and the competent authority to decide what information is commercially sensitive and therefore confidential. However, notifiers must still provide the following information - the general characteristics of the GMM, the name and address of the notifier and location of use, and an evaluation of the foreseeable effects, particularly any pathogenic and/or environmental effects.

The new Directive removes the requirement to state the purpose of the contained use and the methods and plans for monitoring, but notifiers must state the class of contained use and the measures of containment. Emergency plans, in theory, should be *'...supplied in an appropriate manner and without them having to request it, to bodies and authorities liable to be affected by the accident. The information shall also be made publicly available'*.

7.1.7 Liability Clause

Although the European Parliament wanted to include a clause which would have established liability for damage arising from the use of a GMM, the Commission rejected this amendment on the grounds that it wanted to deal with liability and liability insurance 'in a horizontal manner' across all EU legislation instead of piecemeal in individual directives. However, this issue was raised when the Directive was first drafted 10 years ago and was rejected on the same grounds. There is still no legislation to deal with liability 'in a horizontal manner'.

There is extensive provision for commercial confidentiality to be claimed to avoid public disclosure

7.2 The UK's Proposals for Changes to Regulations

The new Directive presents a real opportunity for the UK Government to improve the current regulatory framework for GMMs. Because the revised Contained Use Directive only sets *minimum standards*, the UK is free to impose stricter regulations to protect human health and the environment.

The main features of the UK's proposals are that[63]:

- Interim arrangements mean that some information on all centres using GMMs will be included in the public register. This goes a small way to addressing the disturbing lack of information currently available.
- GMMs will be placed in a class (1-4) which will determine the containment level depending on a risk assessment. This removes the division between large and small scale use (i.e. Group I and II) and is easier to understand.
- Plasmids and naked DNA are not included in the scope of the Regulations so need not be considered in risk assessments.
- Inactivation of waste (from all classes) before release is required, although live GMMs could still be released as inactivation does not mean that all organisms will be killed.
- It is accepted that *limiting* (not *preventing*) contact of GMMs with the environment is sufficient because *"...absolute prevention of contact at the lower levels of containment (i.e. levels 1 and 2) is neither possible nor necessary on safety grounds"* (p 19).
- There will be improvements to the format of information on the public register but no dates of starting or finishing projects will be included.
- There is extensive provision for commercial confidentiality to be claimed to avoid public disclosure. The details that must be included are the name and address of the notifier, the location of the activity, the general characteristics of the organism (only a general description such as bacteria, yeast, or virus may be required), the class of activity, containment measures, waste treatment and the risk assessment. Details of the genetic modification and much other information - including monitoring plans - can be claimed to be confidential.
- The requirement for annual returns is removed so even less information will be collected about the ongoing use of GMMs.
- Notifiers or users of GMMs continue to be responsible for the classification of GMMs.
- Only notification of the first use of a Class 1 organism is required.

Notifiers or users of GMMs continue to be responsible for the classification of GMMs

Only notification of the first use of a Class 1 organism is required. Subsequent uses can take place without notification

Subsequent uses can take place without notification.

- The use of Class 2 organisms has to be notified. However, no consent is required after the first use is approved.
- The use of Class 3 and 4 organisms can only take place with explicit consent.
- Risk assessments of Class 1 GMMs remain in the hands of the user with no obligation to notify the HSE, making scrutiny difficult. If a GMM is erroneously placed in Class 1 when it is actually of higher risk and should be in higher containment, it may be released to the environment.
- In the risk assessment, no consideration is given to the justification for undertaking a particular genetic modification. This leaves workers and the environment unjustifiably vulnerable should accidents occur following irresponsible experimentation.
- No system of independent monitoring is introduced. Such a system is allowed for in the current Irish regulations and should have been included. Inactivation of waste does not kill all organisms and independent scrutiny and setting of legal limits should be required.

No system of independent monitoring is introduced

- Users may apply for dispensations from treating waste before disposal of low risk organisms.
- Allowance is made for certain GMOs and GM techniques to be exempt from the regulations. The HSE estimate that this could involve as many as 400-500 new projects annually.
- The use of GM animals and plants is included but only if the genetic modification results in an increased likelihood of damage to human health. Therefore, the only requirement for environmental risk assessment of the contained use of GM crops and plants remains under regulations from the Environmental Protection Act of 1990, which require the user to undertake a risk assessment and keep records but not to inform any authority, gain approval or be scrutinised.

The main benefits identified from the new regulations "…are expected to take the form of cost savings to centres using GMOs"

The main benefits identified from the new regulations *"...are expected to take the form of cost savings to centres using GMOs"* [63]. However, there will be few benefits for human health and the environment because, overall, the regulations have been weakened. There is the possibility that large scale releases of GMMs could begin with no framework for imposing standards or monitoring.

8. CONCLUSIONS

The use of GMMs for research and for the commercial production of enzymes and drugs has become commonplace. Ensuring that they are used safely is of prime importance. An effective regulatory system requires:

- knowledge of what activities are taking place;
- a system of risk evaluation which is robust;
- an effective system of monitoring;
- proper policing and enforcement of regulations;
- transparency and openness to public scrutiny.

The present regulatory system has failings in all of these areas. The revision of the regulations provides the opportunity to address these weaknesses but, judging by its proposals, it is an opportunity which the Government is reluctant to take.

No authority has comprehensive information about the large scale, industrial use of GMMs in the UK

8.1 Information about Activities with GMMs

No authority has comprehensive information about the large scale, industrial use of GMMs in the UK. Neither is there comprehensive information about research activities. Centres are registered with the HSE but, once registered, do not have to inform the HSE as long as *in the opinion of the user* they are only using low risk, Group IA or B GMMs.

This means there is no overall picture of the use of GMMs, where they may be released accidentally or intentionally, what products they are being used to produce or if there is the potential for recombinations between GMMs to take place in mixed waste. Because there are no data about start and finish dates of projects, knowledge of what is happening at any one time is impossible.

The proposed interim arrangements for the new regulations go part way to addressing this lack of information by requiring all centres to re-register and so very basic information will be entered on the database. However, data on actual organisms used, products made, monitoring, etcetera - especially for GMMs categorised as low risk - will not be included, leaving knowledge about the situation to the subjective impressions of regulators.

Despite the considerable uncertainty surrounding both the health and environmental impact assessments of GMMs, the culture in Government and among regulators appears to be that risk classifications are accurate and correct

8.2 Risk Evaluation

The risk assessment system depends upon being able to place a GMM in a containment class with confidence. Despite the considerable uncertainty surrounding both the health and environmental impact assessments of GMMs, the culture in Government and among regulators appears to be that risk classifications are accurate and correct. This rather unscientific sense of security appears to have driven the lack of scrutiny of discharges and examination of the fate of GMMs.

Because the success (in terms of ensuring safety) of the risk assessment process depends fundamentally on the class into which a GMM is categorised, this should

not be left to those who may have a vested interest in having the lowest possible containment for reasons of convenience or finance.

Genetic modifications are now being undertaken which could make organisms much more pathogenic and/or enable them to infect a wider range of species

The main concerns in relation to human health are that genetic modifications are now being undertaken which could make organisms much more pathogenic and/or enable them to infect a wider range of species. Dramatic changes in pathogenicity can arise through very simple changes which may be unpredictable. On at least one occasion, concern has been expressed that the researchers had an unrealistically optimistic view of the risks[54].

In assessing the potential for environmental harm, the situation appears even worse than for human health risks. Only a cursory approach is taken to environmental risk assessment. However, there is evidence that even 'low risk' GMMs can persist for days if not weeks in the environment, that safety mechanisms to prevent gene transfer by plasmids are not 100% effective, that gene flow between micro-organisms is ubiquitous in nature and that naked DNA can be taken up and incorporated into micro-organisms. Understanding of microbial ecosystems is extremely poor. Therefore, if a rigorous view of the scale of the uncertainties is taken, glib conclusions that any GMM poses no risk to the environment are not scientifically defensible.

No experiment with a GMM can be stopped on the grounds that there is no scientific justification for its creation or because it is irresponsible

No experiment with a GMM can be stopped on the grounds that there is no scientific justification for its creation or because it is irresponsible. The only conditions which can be imposed relate to the class of confinement. As no containment system can ever be 100% effective, risks may be taken for which there is no broader social benefit in terms of the acquisition of scientific knowledge.

The proposed new regulations leave most of the risk assessment in the hands of the users with little formal scrutiny for the majority of uses. Introducing safeguards on the quality of risk assessments is vital.

8.3 Monitoring

The absence of any independent monitoring is one of the most strikingly obvious shortcomings of the present situation. In contrast to the regulation of chemical discharges from factories, for example, there is no requirement either for the user, the HSE or the Environment Agency to monitor releases.

The absence of any independent monitoring is one of the most strikingly obvious shortcomings of the present situation

A circular argument is often used to justify a lack of monitoring. This is based on an assessment that the organism itself is 'safe' and, therefore, there is no need to monitor for it and no real concern about safety. However, this means that no data can ever be collected which questions the original assumption that the GMM is safe, even though such assumptions are subject to huge uncertainty and ignorance about the potential impact of GMMs and their transgenes.

Although monitoring is not easy and combinations of methods will be needed, this argues for an investment in their rapid development rather than a failure to monitor at all. The demands of industry for non-GM food ingredients has led to the rapid emergence of sensitive tests for particular DNA sequences. The military

in the US, concerned about the use of biological weapons, are also developing sensitive tests for organisms. There is no reason why such tests could not be developed for use in the monitoring of contained use. Until reliable monitoring systems are established, the routine discharges of GMMs should not even be considered.

8.4 Policing and Enforcement

Serious breaches of the Contained Use regulations have already occurred but these probably represent the tip of the iceberg. Having the equivalent of one person to inspect the activities of about 500 sites is clearly inadequate. Unless there are more resources allocated to inspections, there can be no confidence that safety measures are being observed.

Policing and enforcement should also include setting standards and release limits for GMMs with the default level being zero. It is only by doing this that there will be the impetus to monitor routinely. Not only is this the standard approach to pollution regulation in the UK, it is used in practice in Denmark and has the additional advantage of allowing for prosecution should limits be exceeded.

8.5 Transparency and Openness to Public Scrutiny

Why should the activities of researchers and companies involved in the use of GMMs be secret?

Secrecy breeds suspicion. The use of GMMs is shrouded in secrecy and obtaining information is difficult, takes a long time and can be expensive. To obtain a list of projects requiring containment levels 3 and 4, GeneWatch was charged £50 by the HSE and a further £25 to obtain additional information about the Group II research activities of industry. Information initially requested on 4th January 1999 was not provided at the time because several companies withdrew their notifications. However, an application for the data under the Environmental Information Regulations resulted in the information arriving in May 1999 - some four months after the original request.

Nor is it easy to access even the limited data in the public registers since these are located in London and Bootle and no data is available via the Internet.

Allowances for commercial confidentiality dominate the current regulations and remain in the proposed revised regulations. Why should the activities of researchers and companies involved in the use of GMMs be secret? There is a wider public interest in being informed both to allow scrutiny of the nature of what is taking place and to help avoid harm arising.

9. RECOMMENDATIONS

To address the issues identified in this report, the following measures should be included in the new regulations.

More information must be obtained:

1. The HSE must backdate the public register to pre-1992 to include *all* centres registered as using GMMs. Information on the commercial use of GMMs must be collected and include data on the products manufactured from them. The proposed interim arrangements should be extended to include this.
2. Annual returns must be continued and extended to include lists of all risk assessments undertaken to enable scrutiny of the evaluations conducted by users of GMMs.
3. The public register must be made available via the Internet, should include a search engine and be comprehensive. Information must include details of the organisms involved, how they are modified, why the modification is being undertaken, how the risk assessment has been arrived at, the dates use started and finished, what precautions are being taken to prevent release, and what monitoring takes place.

In taking decisions about GMMs - and given the uncertainties involved and the potential for serious irreversible harm - a precautionary approach must be adopted

Risk evaluations must be improved:

4. In taking decisions about GMMs - and given the uncertainties involved and the potential for serious irreversible harm - a precautionary approach must be adopted.
5. Plasmids and naked DNA should be brought within the scope of the regulations.
6. Users must be required to present a worst case scenario when notifying the use of a GMM to reveal the full extent of the uncertainties.
7. The requirement for physical barriers to the release of GMMs should remain, together with the presumption (for all classes of GMMs) that there should be no releases of living GMMs into the environment. No discharges should be allowed unless reliable monitoring is available, a detailed risk assessment is presented which takes into account the local environment and the use of other GMMs, and a full justification for the need to discharge live GMMs or intact DNA is given.
8. Provisions for liability for any environmental harm arising from the use of GMMs should be included in the new regulations.

Pollution from GMMs must be monitored, policed and appropriate controls enforced:

There should be no releases of living GMMs into the environment

9. The development of effective monitoring techniques must be a priority.
10. A legal system specifying the levels of GMM pollution that can be released in waste should be established. This would be consistent with other approaches to pollution control (e.g. chemicals), allow for prosecutions if breaches arise and drive a proper monitoring system.
11. The Environment Agency should be made responsible for independent monitoring of environmental releases of GMMs via waste streams and air and for the policing of discharges.

12. In addition, users of GMMs must be required to monitor to verify containment procedures and to implement systems for the detection of sudden leaks.
13. There must be increased investment in policing and enforcement.

Openness and transparency of the regulatory system must be established:

14. Refusal to disclose information about releases of GMMs to the environment on the grounds of commercial confidentiality must not be allowed under any circumstances. Users must supply details of any GMMs (including the species and how and why they have been genetically modified), the levels of release to the environment in waste and the monitoring systems in place.
15. Representation of public interest groups should be increased on the advisory committees, meetings should take place in public, and annual reports summarising each year's activities should be produced.
16. There should be greater public involvement in decision-making about the use of GMMs.

Refusal to disclose information about releases of GMMs to the environment on the grounds of commercial confidentiality must not be allowed under any circumstances

APPENDIX 1: ADVISORY COMMITTEE MEMBERSHIP

Members of the Advisory Committee on Genetic Modification (May 1999)

Professor K Davies	(Chairman)	University of Oxford
Professor J Beringer		University of Bristol
Professor T M Roberts		Institute of Terrestrial Ecology
Mr S Vranch		Jacobs Engineering
Mrs D Carey	(TUC nominee)	Institute of Virology
Dr J Kinderlerer	(TUC nominee)	University of Sheffield
Dr R Owen	(TUC nominee)	TUC Medical Advisor
Mr R Spiller	(TUC nominee)	MSFU
Dr K Edwards	(CVCP nominee)	University of Leicester
Dr M Gale	(Research Councils' nominee)	John Innes Centre
Professor S Hughes	(CBI nominee)	University of Exeter

Members of the Technical Sub-Committee of the Advisory Committee on Genetic Modification (May 1999)

Mr S Vranch	(Chairman)	Jacobs Engineering
Professor D Young		Imperial College
Dr J Marshall		University of Oxford
Mr S Eley		Defence Evaluation and Research Agency
Professor A Minson		University of Cambridge
Dr M Mackett		Christie Hospital, Manchester
Dr P Hirsch		Institute of Arable Crops Research
Dr P Minor		National Institute for Biological Standards and Control
Professor D Onions		Glasgow University
Professor D Jeffries		St Bartholomew's Hospital
Dr R Randall		University of St Andrew's
Dr I Cooper		Institute of Virology and Environmental Microbiology
Mr J Thorley	(CBI nominee)	Consultant
Dr J Kinderlerer	(TUC nominee)	University of Sheffield

APPENDIX 2: REGISTERED LARGE-SCALE GMM USERS

Centres registered as undertaking large scale work with GMMs and their reply or comments in response to a letter and follow-up phone calls from GeneWatch requesting further information about their activities and monitoring arrangements.

CENTRE NAME AND ADDRESS	REPLY
BRF Lyttel Hall, Nuffield, Redhill Surrey RH1 4HY	None
Carlsberg Tetley Brewing Ltd 107 Station Street, Burton upon Trent, Staffs, DE14 1BZ	Used for testing GM yeast in beer production - not in commercial use.
Celltech Therapeutics Ltd, 216 Bath Road, Slough, SL1 4EN	Have sold their commercial production division using GMMs to Lonza Biologics
Chiroscience Group Plc Holmewood Hall, Holme, PE7 3PG	Use large scale but claimed commercial confidentiality
Delta Biotechnology Mabel Street, The Meadows, Nottingham NG2 3ED	None
Delta Biotechnology 5 Crocus Street, The Meadows, Nottingham NG2 3DE	None
Delta Biotechnology Ltd Castle Court, 59 castle Boulevard, Nottingham NG7 1FD	None
Eli Lily & Co Ltd Fleming Road, Speke, Liverpool, L24 9LN	None
Genzyme Biochemicals 50 Gibson Drive, King's Hill, West Malling, Kent ME19 6HG	None
Hoechst Roussel Vet Ltd Walton Manor, Milton Keynes MK7 7AJ	None
Imperial Biotechnology Ltd Southbank Technopark, 90 London Road, London SE1 6LN	None - could not locate for follow-up call.
Lonza Biologics Plc 226/228 Bath Road, Slough, SL1 4DY	None
Medeva Pharmecuticals Ltd Gaskill Road, Speke, Liverpool, L24 9GR	None
MRC Cell Mutation Unit University of Sussex, Falmer, Brighton, BN1 9RR	No large scale IB use - small scale only, no commercial.
Murex Biotech Ltd Central Road, Temple Hill, Dartford, Kent, DA1 5LR	None
Pfizer Central Research Ltd Ramsgate Road, Sandwich, Kent, CT13 9NJ	None
Queen Mary & Westfield College University of London, Mile End Road, London E1 4NS	Research only, no commercial.
Quest International Menstrie, Clackmannanshire, FK11 7ES	Research use only, no commercial.

Continued overleaf...

Continued…

CENTRE NAME AND ADDRESS	REPLY
University of Newcastle upon Tyne School of Biological and Nutritional Sciences, Newcastle-upon-Tyne, NE1 7RU	None
Sigma Aldrich Co Ltd Fancy Road, Poole, Dorset, BH12 4NZ	None
Smithkline Beecham Coldharbour Road, The Pinnacles, Harlow, Essex CM19 5AD.	None
Smithkline Beecham Shewalton Road, Irvine, Ayrshire, KA11 5AP	None
Smithkline Beecham Pharmaceuticals Great Burgh, Yew Tree Bottom Road, Epsom, Surrey, KT18 5XQ.	Closed
Smithkline Beecham Pharmaceuticals Clarendon Road, Worthing, West Sussex BN14 8QH.	None
Tate and Lyle Citric Acid Denison Road, Selby, North Yorkshire, YO8 8EF.	No large scale use
Unilever Research Colworth Laboratory Colworth House, Sharnbrook, Bedfordshire, MK44 1LQ.	No large scale use
University of Cambridge Hills Road, Cambridge, CB2 2QL	None
University of Cambridge Tennis Court Road, Cambridge, CB2 1QT.	None
University of Coventry Biology Department, Priory Street, Coventry CV1 5FB	None
University of Cranfield Biotechnology Department, Cranfield, Bedfordshire, MK43 0AL	No IB since 1989 and never commercial
University of Westminster 115 Cavendish Street, London W1M 8JS	No commercial use large scale pre 1992
Zeneca Agrochemicals Limited Jealott's Hill Research Station, Bracknell, Berkshire, RG12 6EY.	None
Zeneca Bioproducts PO Box 2, Belasis Avenue, Billingham Cleveland TS23 1YN	None
Zeneca Pharmaceuticals Alderley Park, Macclesfield, Cheshire, SK10 4TG	None

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