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GeneWatch UK

The Courtyard, Whitecross Road, Tideswell, Buxton, Derbyshire, SK17 8NY, UK
Phone: 01298 871898 Fax: 01298 872531 E-mail:gene.watch@dial.pipex.com

Website: http://www.genewatch.org

GENETICALLY MODIFIED MICRO-ORGANISMS:
Leaking from the Lab?

GeneWatch UK

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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 deliberately released into the environment.

However, contained use GMMs are also being discharged into the environment either through the breakdown of containment facilities or through routine discharges if the GMM is deemed 'safe'. There is no monitoring or policing of such discharges and no requirement to label products made using GMMs if the GMM itself is not in the product. This briefing examines what is known about the use of GMMs in the UK and whether current safety systems are adequate.

The Use of GMMs in the UK

There are approximately 500 sites using GMMs in the UK although the precise number is unknown because prior to the introduction of the 1992 Contained Use regulations this information was not included on the public register.

The use of GMMs on a small scale is mainly for medical and other scientific research. Inevitably, the vast majority of research with GMMs - both in commercial and public facilities - is being undertaken into diseases in humans with a smaller amount of work concerning disease in domestic animals. The work being undertaken is very varied but includes:

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

In industrial laboratories, the production of chemicals/drugs is another important research dimension which includes:

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

The kinds of products which are already being produced commercially using GMMs include enzymes for food processing and detergents (e.g. chymosin), food additives (e.g. aspartamate), and pharmaceuticals (e.g. insulin).

Risks of Releasing GMMs

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.

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

Many of the GMMs used in contained facilities have either been bred in laboratories over many generations and, in theory, lost their ability to survive in the natural environment or have had specific sequences inserted or deleted to reduce their ability to survive. However, there is evidence that these disabled organisms (such as *E.coli* K12, one of the organisms most commonly used in GM experiments) 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, for instance, various strains of *E.coli* K12, including GM versions, survived for up to 7-14 days but did not appear to colonise the intestine[1,2,3,4]

The Health and Safety Executive (HSE) guidelines on risk assessment state that *E.coli* K12 can survive for 7 days in external environments[5]. However, other research indicates that this may be an underestimate in some circumstances although there is great variation between studies, probably related to differing experimental conditions. For example, *E.coli* K12 in a small sludge unit could not be detected for 12 days but then 'reappeared'[6]. Other research has shown that a GM *E.coli* K12 strain survived for at least 35 days in a non-sterile silt loam soil[7]. In contrast, in other studies a BST-producing (bovine somatotrophin) strain of *E.coli* K12 was eliminated from sewage sludge over 5-6 days[8].

GMMs may not only survive in water, soil or air, they may also be ingested by organisms living in soil. Experiments have shown that a GM bacteria, *Pseudomonas fluorescens*, can survive and multiply in the intestines of the earthworm, *Octolasion cyaneum*[9], and the woodlouse, *Porcellino scaber*[10]. Because these organisms are consumed by others, GMMs could move through the food web.

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 distinguish 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.

Conclusions

Disturbingly, there is no overall picture of the use of GMMs, where they may be released accidentally or intentionally, and what products are being developed from them. Centres using GMMs are registered with the HSE but, once registered, do not have to inform the HSE of any new uses as long as *in the opinion of the user* they are only using low risk, Group I GMMs.

Existing safety regulations depend on the accurate determination of a GMM's risk assessment category. Yet, 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 complacent and unscientific sense of security appears to have been responsible for the lack of scrutiny of releases of GMMs.

Until reliable monitoring systems are established, the routine discharges of GMMs should not even be considered

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. A circular argument is often used to justify this lack of monitoring – because the organism is 'safe' there is no need to monitor for it. However, this means that no data can ever be collected which questions the original assumption that the GMM is safe.

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 the monitoring of contained use and until reliable monitoring systems are established, the routine discharges of GMMs should not even be considered.

Because GMMs are living organisms, mistakes will not be rectifiable once any harmful effects have become apparent

Policing and enforcement should also include setting standards and release limits for GMMs with the default level being zero. 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. Because GMMs are living organisms, mistakes will not be rectifiable once any harmful effects have become apparent. It is therefore essential to adopt a precautionary approach in order to avoid irreversible damage to health and the environment.

No limits are set for the number of GMMs which can be released and there is no policing of discharges

In their applications to the HSE to register uses of GMMs, companies have acknowledged that releases will take place even if waste is 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 (10^6 is one million organisms). 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 at 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.

Despite the lack of knowledge about how GMMs behave in the environment, the HSE has not carried out any routine, independent monitoring as there is no requirement in the regulations to do so. No limits are set for the number of GMMs which can be released and there is no policing of discharges.

Revising the Regulations

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

Overall, the regulations will be weakened

A revised Directive has now been agreed[20] and has to be implemented by Member States by 5th June 2000. The HSE has therefore recently begun a consultation process to determine how the UK regulations should be amended[21]. Because the European Directive sets out *minimum* standards, Member States may introduce tighter regulations. However, current proposals suggest that this is unlikely to be the case in the UK where the main benefits identified from the proposed new regulations *"…are expected to take the form of cost savings to centres using GMOs"*[19]. At the same time, there will be few benefits for human health and the environment because, overall, the regulations will be weakened[22]. In particular, the failure to impose maximum release limits and monitoring requirements means that large scale releases of GMMs could become routine.

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. Evidence suggests that transformation can take place both in aquatic and terrestrial environments[11,12,13]. Whilst the frequencies of such transformation events may be low, making detection difficult, 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.

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

Conjugation: Movement of DNA between bacteria following cell-to-cell contact and effected by plasmids or transposons. One of the most important safety mechanisms in the production of GMMs is the use of plasmids which are deficient in gene transfer mechanisms and have a restricted host range. However, research has shown that such plasmids and transposons can acquire the ability to transfer genes from other organisms, which has led to the observation that *"there is no such thing as a safe plasmid"*[14].

Transduction: The transfer of genetic material from one bacterium to another by a bacteriophage (a virus which infects bacteria). Although there is evidence that a large number of phages exist in the environment, there are little data about the frequency of transduction in the wild and thus its significance for GMMs is difficult to assess.

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

- **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 drug resistant disease if the genes pass to other organisms. Although such genes are ubiquitous in nature, 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[15]. Conceivably, so called 'pathogenicity islands', which are regions of DNA that contain a variety of virulence genes[16], could be transferred.
- **The introduction of genes from vectors (the plasmids, transposons and phages used in genetic modification) which facilitate the transfer of foreign DNA into an organism which would otherwise reject it.** In naturally occurring organisms, certain 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[17].

Although safety mechanisms may be built into GMMs, they are by no means foolproof

Safety Regulations

The HSE has estimated that about 5,500 new projects with GMMs are undertaken each year

Current UK regulations implement the European Union's Contained Use Directive (90/219/EEC) and specify the degree to which a GMM should be prevented from escaping to the environment (its 'containment level'). This is dependent on an assessment of whether the GMM poses a low or higher risk to human health or the environment (Group I or II), together with the scale of its use (Type A or B).

There are four risk assessment categories to establish containment levels:

- **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).

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[18]. Once registered as a Group I centre, there is no requirement to inform the HSE of any subsequent uses of other GMMs if the user decides that these are also low risk. However, the HSE has estimated that about 5,500 new projects with GMMs are undertaken each year, 90-95% of which are Group IA[19].

Risk assessments of GMMs are largely the responsibility of the user and are rarely scrutinised by the HSE

Although the risk assessment of GMMs is critical to the implementation of safety regulations, this is largely the responsibility of the user and their assessments are rarely scrutinised by the HSE. As the responsible agency in the UK, the HSE does however carry out inspections of GMM sites but these are limited in scope and frequency since, in terms of hours allocated, there is only the equivalent of one person to visit approximately 500 sites. Even so, the HSE has taken action against seven institutes and universities for breaches of safety procedures since 1992 (see Table 1).

All users of GMMs covered by the Contained Use 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 measures include air filtration systems, protective clothing, and the ability to fumigate and isolate premises to prevent a GMM physically escaping. Biological containment involves changes to the organism which mean that if it does escape it has a reduced ability to survive, cause disease or other harm. 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.

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

The HSE has taken action against seven institutes and universities for breaches of safety procedures since 1992

Releases of GMMs into the Environment

The HSE have said that, in their experience, large scale facilities routinely inactivate all GMMs prior to disposal. However, there is no explicit legal requirement to kill all GMMs if they have been deemed 'safe' and are considered to have a limited ability to survive in the environment (Group I organisms). Therefore, living GMMs will be entering the environment in waste from both industrial and research facilities using these organisms. Although waste from research facilities may be heat treated in autoclaves or chemically treated before disposal by methods such as incineration, there is no requirement for this and no independent verification that this is undertaken.