Twelve months after Séveso

A YEAR AGO last Sunday, Séveso 'happened'. A trichlorophenol reactor at the Icmesa chemical plant there spewed its contents over the surrounding area. They included about 1 kg of a highly toxic by-product, 2,3,7,8-tetrachlorodibenzo-paradioxin, known as dioxin. Controversy has raged ever since. It has involved both the plant's Swiss owners (Givaudan, a subsidiary of Hoffman-La Roche) and the Italian authorities; the scientific, legal and other aspects of the disaster; and, above all, the many people affected directly or indirectly by the dioxin. Séveso has received probably the most extensive media coverage ever accorded to the site of an industrial accident, and is a watchword for pollution.

Amidst all this two facts about the disaster are irrefutable, their consequences disastrous. The first was the venting of the reactor discharge directly to the atmosphere, an obvious fault of design. The second was the two-week delay in evacuating residents from the area, responsibility for which cannot with certainty be established but is part of the deliberations of the Italian court investigating the accident. For the people exposed to the dioxin, the health picture shows 106 children with the skin disease chloracne, but no evidence of immunological damage; no evidence of malformation attributable to the dioxin in the foetuses legally aborted from Séveso women exposed to the chemical, and no evidence of chromosomal damage in these foetuses; and a number of children born with abnormalities in the Séveso region which is reported to be no greater than the statistical norm for the area.

The plan to monitor the health of everyone exposed to the dioxin, though medically and politically necessary, will not be a true epidemiological survey of 'dioxin risk'. A more selective survey of categories of 'high, low and non-risk' groups is under review and will yield results more rapidly. There are fewer grounds for optimism over the question of decontamination policy for the removal of the dioxin. The section of Séveso which housed most of the evacuated residents has been decontaminated by the plant's owners and pronounced safe for habitation. But the methods to be adopted for the remainder of the contamination area are still disputed.

What are the wider implications of Séveso? The issue of reform of the abortion laws in Italy is one. Another concerns one of the proposals of the 1976 World Health Assembly which advocated that WHO collate information on birth defects in the populations of member countries; the collation is now more urgent than ever. The lessons for the general public, the chemical industry and governments are obvious. There are dangers involved in the operation of chemical plants. And the cost of disasters in the industry is great. Governments need to keep their pollution control measures under constant review. The fact remains, however, that it takes a major disaster to make things happen; and that has been of little comfort to the people of Séveso over the past 12 months. □

Rational containment on recombinant DNA

The US National Institutes of Health has recommended that all recombinant DNA experiments, involving any animal virus, should be subject to the highest containment conditions, even though other work with the intact virus may require less stringent conditions. Lennart Philipson, of the Department of Microbiology, Uppsala University, Sweden, and Pierre Tiollais, of the Institut Pasteur, Paris, put forward the case for categorising recombinant DNA experiments involving animal viruses more rationally.

THE development of molecular genetics has depended largely on the detailed analysis of bacterial viruses, the bacteriophages, and their use to transfer bacterial genes between bacteria which do not normally recombine. Animal viruses now promise to provide a similarly detailed insight into the molecular mechanisms of gene expression in animal cells, which will undoubtedly contribute to an understanding of man and his domestic animals both in health and disease and, it is hoped, contribute to the development of medical and veterinary science.

Restriction enzyme mapping of the viral genome enables regions of interest to be excised and analysed. In many cases the full development of this work requires the use of recombinant DNA techniques to purify and clone the resulting portions of the viral genome. Animal viruses can also be used as vectors for introducing other eukaryote DNA into animal cells in which its expression may be studied. At present, however, the guidelines on recombinant DNA research laid down by the US National Institutes of Health (NIH) require almost all recombinant DNA experiments involving animal viruses to be carried out under the highest containment conditions. In practice these requirements preclude most experiments.

Animal viruses as a class are unfortunately regarded by the general public and the uninformed scientific community as rather mysterious entities which cause pandemics, epidemics and isolated cases of dreadful infectious diseases against which there is no remedy. Scientists themselves have coloured this picture by suggesting that viruses may cause cancer in man, implying to the layman that cancer is an infectious disease. In reality, animal viruses are extremely diverse; some are known to be highly infectious and pathogenic. Others seem to be harmless. It should therefore be mandatory to discriminate between different viruses according to their pathogenicity to man and other mammals when formulating guidelines for the use of viral genomes in *in vitro* recombinant DNA research.

The conjectural hazards envisaged arise from the (hypothetical) risk that virus genomes, when introduced into bacteria or when used as vectors for introducing DNA into mammalian cells, may give rise to "new infectious entities" or may transfer their pathogenic or tumour-producing capacity from the new host back to man or other mammals. Both types of risk depend on chains of events with low probabilities.

Against these arguments it should be considered, first, that the transfer of all or part of the viral genome to bacterial cells may not be an entirely new event. For millions of years prokaryotic organisms have been exposed to eukaryotic DNA and several microorganisms have efficient systems of transferring DNA over recombinant barriers. Second, the development of a 'new infectious entity' would probably require expression of the viral genes in the prokaryotic cell. This must have a low probability since most genes from eukaryotes and viruses, which have been transferred up till now, are not faithfully expressed as RNA or protein in bacteria[1–4]. Even if the inserted viral genes are expressed the resulting risk would not be greater than that from production of viral proteins in cell cultures, since only fragments of the viral genomes will be inserted in the experiments proposed.

The conjectural hazards may therefore be confined to the transfer of viral nucleic acids from the modified bacterium to an animal or human host. This would require a chain of events, each of low probability: the bacterium containing hybrid DNA must first escape from the laboratory and then establish itself in a new host. The viral nucleic acid must then enter the cells of the new host, and there express its tumorigenic or other harmful properties. Even without considering containment we are probably discussing probabilities in the range 10^{-20}–10^{-28} for the transfer of harmful genes into the new host.

Against this background it is obviously necessary to prohibit experiments with viruses such as Lassa fever virus or Marburg virus, which are considered to be high risk viruses in diagnostic laboratories; but it is not clear that viruses classified as low risk, such as adeno, SV40 and polyoma viruses, need such high containment. SV40 virus has already been injected accidentally together with polio vaccine into millions of human subjects without any registered harmful effects[5,6], although antibodies against SV40 appear in the serum. Some adenoviruses infect humans readily and about 50–80% of the population develop antibodies to adenovirus types 1 and 2 from the age of 5–10 years[7]. Even the adeno–SV40 hybrid viruses which were originally isolated when adenovirus vaccines were developed in monkey kidney cells have been introduced into large groups of military recruits as an inactivated or live vaccine without any detrimental effects[8,9]. Careful studies have failed to find an association between adenovirus transcripts, T antigens or other adenovirus products with tumours in humans[10,11].

Normal biochemical work with adeno and SV40 viruses is considered to fall into the low risk category under guidelines published by the National Cancer Institute and those from the Center for Disease Control, Atlanta, Georgia. These regulations allow the use of large amounts of virus. Milligram quantities of viral DNA are handled regularly by investigators and technical personnel in several biochemical laboratories. Provided that expression of virus DNA is prohibited in the bacterial cell, which is likely (see above), it must be more risky for personnel to handle viral DNA than to insert it in a bacterial vector. The NIH guidelines request P4 and EK2 conditions to handle partial or intact viral DNA in a prokaryotic host although as we have pointed out the risks envisaged are comparable to or lower than those encountered when working with intact virions. It is possible to claim that virions present a higher risk since as long as the protein coat is present the virion may possibly escape from the laboratory and infect humans. Thus, there seems to be a disproportionately high containment requirement for work with animal virus genomes in bacteria.

It is also difficult to understand the containment requirements for work with eukaryotic vectors. In the case of SV40 it has been established that SV40 DNA will form hybrids with cell DNA when cells are infected at high multiplicity[12,13]. Such experiments are in essence shotgun experiments, but since no *in vitro* recombination is involved it is considered a low risk experiment requiring no containment facilities. If a similar experiment is carried out *in vitro* between DNA from the same cell and SV40 virus DNA (probably with lower efficiency), it is labelled as P4 according to the NIH guidelines. Insertion of SV40 sequences into adenovirus DNA also occurs frequently when the two are cultivated together, as exemplified by the adeno–SV40 hybrid viruses[14,15] and new hybrids recently developed (J. Sambrook and G. Fey, personal communication). To take advantage of the natural recombination between SV40 and adenoviruses requires only a moderate risk containment facility, but the NIH guidelines absolutely forbid these experiments using recombinant DNA techniques. Here there is also a distinct discrepancy between the guidelines and other regulations for the study of animal viruses.

Most of the confusion is probably due to the fact that natural and artificial recombinant DNA reserch has not been considered as a whole. Only the new *in vitro* recombinant DNA technique has formed the basis for developing the NIH guidelines, although guidelines for work with dangerous microorganisms have been in existence for a long time. Consistent rules for all recombinant DNA research are therefore needed.

A good definition of recombinant DNA research might be that suggested by the Standing Advisory Committee on Recombinant DNA of the European Molecular Biology Organisation (EMBO): recombination of DNA molecules of different biological origin by any methods that overcome generally recognised natural barriers to mating, infection and recombination, to yield molecules that can be propagated in some host cells and the subsequent studies of such recombinant DNA molecules. *In vitro* recombinant DNA research is only a subsection of this definition. Within the broader meaning of the term there are several experiments now carried out in low risk containment according to generally accepted guidelines, which must be analysed and compared with the *in vitro* techniques before any regulatory guidelines are issued. It is, for example, possible to transfer the genome of several animal viruses into the capsid of unrelated viruses[16–19], and thereby increase the host range of the viral genes. It is also possible to fuse animal cells, which may involve undesirable transfer of viral genomes to new hosts[20,21]. Many of these experiments involve conjectural hazards comparable to those implied by the *in vitro* recombinant DNA technique.

But in these cases the experiments also probably carry only a low risk if they involve low risk viruses, since similar events probably occur in nature. Therefore we would like to propose that the entire area of recombinant DNA research should be re-evaluated. Experiments which are considered to involve real and proven risks should be prohibited at present. The transfer, by any method, of high risk viral genomes, potent bacterial toxins and antibiotic resistance to gonococci and streptococci fall into this category. Conjectural hazards which may or may not become real should rapidly be evaluated under secure conditions. Meanwhile *in vitro* recombinant DNA research can probably proceed in several areas including the transfer of low risk viral genomes to prokaryotic cells and the development of eukaryotic vectors from low risk viral genomes.

In closing, it should be emphasised that both mutagenisation and deletion of a multitude of genes in the laboratory have, to the best of our knowledge, never provided a selective advantage for an organism in the natural environment. Some genetic manipulation with plant cells may be an exception. It is necessary for the opponents of recombinant DNA research to provide examples of such selective advantage otherwise this debate will focus more on faith than science. □

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