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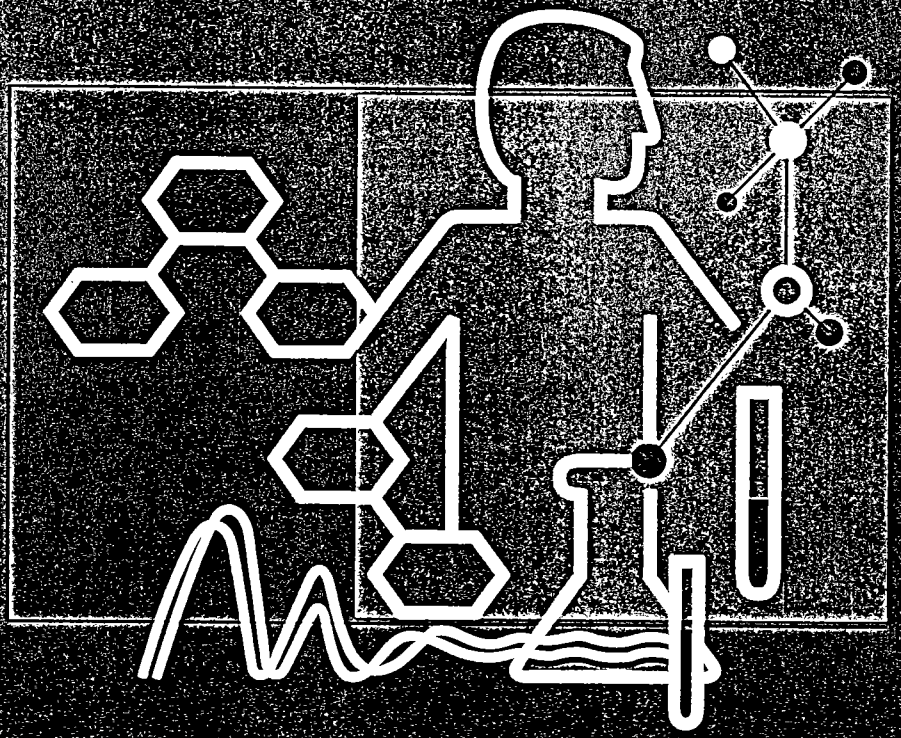
INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

Training Module No. 2

LABORATORY HANDLING OF MUTAGENIC AND CARCINOGENIC PRODUCTS



UNEP

IOMC INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS
A cooperative agreement among UNEP, ILO, FAO, WHO, UNIDO, UNITAR and OECD

WORLD HEALTH ORGANIZATION

IPCS

Training Module No 2

LABORATORY HANDLING OF MUTAGENIC AND CARCINOGENIC PRODUCTS

Translated by Alison Grant, Sevenoaks, United Kingdom, from *Manipulation des Produits Mutagènes et Cancérogènes au Laboratoire*, prepared by the Société Française de Toxicologie Génétique under the coordination of Professor D. Marzin, Institut Pasteur de Lille, France. Financial support for the translation was provided by the International Labour Office.

Produced under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation and the World Health Organization, and within the framework of the Inter-Organization Programme for the Sound Management of Chemicals

The International Programme on Chemical Safety (IPCS), established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organisation (ILO), and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessing the risk to human health and the environment from exposure to chemicals, through international peer-review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) was established in 1995 by UNEP, ILO, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization, and the Organisation for Economic Cooperation and Development (Participating Organizations), following recommendations made by the 1992 United Nations Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities purused by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

S.F.T.G. SOCIETE FRANCAISE DE TOXICOLOGIE GENETIQUE

Laboratory Handling of Mutagenic and Carcinogenic Products

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D. MARZIN AND I. MURANYI-KOVACS

NOTES FOR THE READER

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Nomenclature: *throughout this document the terms mutagenic, genotoxic and carcinogenic are used interchangeably to refer to the products covered by this guide*

The purpose of this document is to provide information on the precautions which should be taken when mutagenic, genotoxic or carcinogenic products are handled; it also indicates the limitations of our knowledge in this field.

It should be emphasized that this work describes the experience of persons who do experimental work in the area of genotoxicity, and **consequently some points are valid only in situations where genotoxic products are handled in research laboratories.** Extrapolation to other fields (organic synthesis, industrial production, medical tests) requires adaptation which can only be done by persons directly involved in those activities.

Use of genotoxic and/or carcinogenic products is not confined to genetic toxicology laboratories alone. There are many laboratories which handle such products:

- biochemistry and molecular biology laboratories (e.g., DNA labelling, revelation and measurement using ethidium bromide);
- genetic laboratories (production of mutants);
- immunology laboratories;
- physiology and pharmacology laboratories;
- chemistry laboratories.

Researchers and technicians who handle these products are often unaware of the risks involved, and this document is intended for them.

In the interests of fuller information we have included in this document the names and addresses of companies supplying equipment or services which improve safety in the handling of genotoxic products. It goes without saying that **neither the Société Française de Toxicologie Génétique (SFTG) nor any member of the working group in any way endorses these companies or has an interest in them.** They are

companies, known to one or more members of the working group, who have used those companies' equipment or services. The working group will be happy to include other references in a new edition, subject to the opinion of the working group which will take efficacy of protection against genotoxic products as its sole criterion for their inclusion.

We plan to update this document at regular intervals.

To help us in this task, readers are invited to send in their comments to the author of each chapter. They will be examined by the working group and the document can then be amended and improved.

Comments which are not on any chapter in particular may be sent to the group co-ordinator (D. Marzin).

II.

INTRODUCTION

D. MARZIN AND I. MURANYI-KOVACS

INTRODUCTION

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Summary:

There are laws in France governing the risks of occupational exposure to chemicals. This legislation encompasses the handling of carcinogenic and mutagenic products but there are no laws specifically on this field. For that one needs to be able to identify the hazardous substances correctly (lists of the European Union and International Agency for Research on Cancer), but other sources too must be consulted, specifically by means of literature searches.

The work published by Ames and co-workers (McCann et al., 1975; McCann & Ames, 1976) first alerted the scientific community to the relationship which exists between mutagenesis and carcinogenesis and provided a practical approach to rapid *in vitro* assessment of the hazard which exposure to mutagens and carcinogens represents.

Following this work, and as our knowledge of the mechanisms of genetic toxicity has developed, the term "genotoxic" has been widely adopted to describe products likely to damage DNA.

Practically speaking it may be said that:

1. The latency period between the start of exposure to a carcinogen and the first appearance of cancerous lesions is usually long (5 to 30 years), which makes it difficult to determine causality.

2. In France in 1991, cancer accounted for 143 267 out of 524 685 deaths (or more than 27% of all causes of death). Authors variously estimate the percentage of cancers attributable to occupational exposure at between 1 and 20%. Doll & Peto (1981) put it at 4% of all cancer deaths in the United States.

1. LEGISLATION

The prevention of occupational risks from the use of chemical and particularly carcinogenic products is now based on a coherent body of laws introduced by **Decree** *(décret)* **92-1261 of 3 December 1992**. This text is both an updating and a reorganization of French legislative texts and it incorporates into French law the latest European directives on the prevention of chemical hazards to workers.

This legislation is grouped in **Section V of Book II, Title III, of the Labour Code – Prevention of chemical hazard** and has eight subsections (Articles R 231-51 to R 231-58).

Subsection I (Art. R 231-51) – Principles for classification of hazardous substances and preparations – is the transposition of European Directive 67/548/EEC of 27 June 1967 on the **classification, packaging and labelling of dangerous substances**, together with its amendments and adaptations. It defines the characteristics of "dangerous" substances which must be labelled, including substances which are toxic, harmful, sensitizing, carcinogenic, mutagenic, teratogenic or hazardous to the environment.

This subsection is relevant primarily to manufacturers and suppliers, ***but for laboratories it means an implicit requirement***

- ***to understand the labelling,***
- ***to label all receptacles into which operators have transferred hazardous products (even where these are diluted)***[1].

Subsection II (Arts. R 231-52 to R 231-52-17) – Reporting of hazardous substances. This section is relevant only to manufacturers and importers of hazardous substances or preparations. It sets out their obligations to provide information to the appropriate authorities.

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[1] The Order (*arrêté*) of 25 November 1993, amending the Order of 21 February 190 defining the criteria for classification and the conditions for labelling and packaging of hazardous preparations (*Journal Officiel* of 7 January 1994) sets out the statutory obligations, taking into account the concentration of preparations (dilutions, mixtures, etc.).
For carcinogens and mutagens in categories 1 and 2 (R 45, R 49 or R 46) there is no longer a labelling requirement if the concentration is less than 1/1000. For carcinogens and mutagens of category 3 (R 40) the statutory labelling requirement stops at a dilution of 1%
The working group believes that this minimalist restriction is not at all appropriate to mutagenic and carcinogenic agents which are already known or currently being tested.

Subsection III (Art. 231-53) – Information on the hazards presented by chemical products.
This requires manufacturers, importers and sellers of hazardous products to supply a **safety information sheet**[2] and states the information this must include, namely:
- identification of the product, identification of the hazards;
- first aid measures, fire control measures;
- precautions to be observed during storage, use and handling
- measures to be taken in the event of accidental dispersion;
- procedures for monitoring workers' exposure and characteristics of adequate personal protection equipment;
- physicochemical properties, stability and reactivity;
- toxicological and ecotoxicological information;
- information concerning transportation and methods of disposing of waste;
- statutory information regarding, in particular, the classification and labelling of the product.

This means that laboratories are entitled to insist that their supplier provides them with safety information sheets in accordance with these requirements.

Subsection IV (Arts. R231-54 to R 231-54-8) – General rules for the prevention of chemical hazard.
This completely new subsection represents the incorporation into French law of European Directives 80/1107/EEC and 88/632/EEC and it makes the general principles of prevention as defined by Law No. 91-1414 of 31 December 1991 (Arts. L. 230-1 to 230-4 of the Labour Code) applicable to chemical risks.

We reproduce below the salient points of this text only.

Art. 231-54 – The basis of prevention shall be a limiting of the use of hazardous products and the number of workers exposed together with the introduction of preventive measures.

Art. 231-54-1 – For every activity which may present a risk of exposure to a hazardous product, the head of the establishment shall prepare… an assessment of the risks run… (which) must evaluate collective and individual levels of exposure and indicate the methods envisaged to reduce them.

Art. R 231-54-2 – Workplaces… shall be fitted with efficient equipment for the removal of vapours, gases, aerosols and dust.

Art. R 231-54-3 – Equipment and apparatus providing collective protection must be regularly inspected and maintained in perfect working order.

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[2] By way of an addition to this subsection, the Order of 5 January 1993 (OJ 7 February 1993) lays down the rules for completing and forwarding safety information sheets and includes a guide to their completion.

Art. R 231-54-4 – Workers shall be provided with means of personal protection appropriate to the hazards encountered.

Art. R 231-54-8 – Suitable safety notices must be placed in work premises where hazardous products are used...[3]

Subsection V (Arts. 231-55 to R 231-55-3) – Monitoring of chemical hazard at places of work.
This requires duly authorized bodies to check that threshold limit values are not exceeded and stipulates the terms on which such bodies are granted authorization.

Subsection VI (Arts. R 231-56 to R 231-56-11) – Special rules for the prevention of carcinogenic hazard.
This new subsection represents the incorporation into French law of European **Directive 90/394/EEC of 28 June 1990** on the protection of workers from the risks related to exposure to carcinogens at work.

Art. R 2231-56 – The provisions of this subsection apply to activities in which workers are exposed or may be exposed to carcinogenic agents in the course of their work. Carcinogenic agents are understood to be any substance or preparation, the labelling of which explicitly mentions its carcinogencity[4], together with any substance, preparation or process defined as such by ministerial order[5].

Art. R 231-56-1 – For any activity which may present a risk of exposure to carcinogenic agents, the employer must evaluate the nature, degree and duration of exposure in order to be able to assess the risk.

All significant exposures, particularly those which may cause skin effects, must be taken into account. The employer must determine the preventive measures to be taken.

This means at the very least that:

- *a list of carcinogenic products used in the laboratory must be compiled and kept up to date;*
- *for each carcinogenic product there must be a sheet describing the risks, pathways of uptake into the body and adequate means of protection;*
- *for every experiment in which carcinogenic products are used, a detailed experimental protocol must be drawn up which states, for every stage at which the carcinogenic agents are involved, the potential risks (individual and collective) and the precise means of prevention or protection which must be used.*

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[3] See Order of 4 November 1993 on safety and health notices at work.
[4] The explicit mentions of carcinogenicity are the risk phrases R 45 and R 49.
[5] Pending new texts, the list in the circular of 15 March 1988 remains applicable.

Art. R 231-56-2 – The employer must reduce the use of a carcinogenic agent at the workplace... by replacing it...

Art. R 231-56-3 – If it is not feasible to replace it... the employer must take measures to ensure that the level of exposure is reduced...

In all cases where a carcinogenic agent is used the employer shall implement the following measures:

a) limitation of the quantities of carcinogenic agent;
b) limitation of the number of workers exposed;
c) **development of working processes and technical measures whereby the release of carcinogenic agents can be avoided or kept to a minimum;**
d) removal of carcinogenic agents in accordance with Art. 232-5-7[6];
e) use of suitable methods for measuring carcinogenic agents;
f) application of appropriate working procedures and methods;
g) collective protection measures or, where exposure cannot be prevented by other means, personal protection measures;
h) hygiene measures;
i) information of workers;
j) **demarcation of hazard areas and use of adequate warning notices in areas where workers are or may be exposed to carcinogenic agents;**
k) provision and installation of emergency equipment;
l) **use of methods whereby carcinogenic products can be safely stored, handled and transported, notably the use of hermetically sealed receptacles which are clearly, distinctly and visibly labelled**[7];
m) safe collection, storage and removal of waste.

Art. R 231-56-5 – Workers must be informed by the employer of incidents or accidents which may have subjected them to abnormal exposure.

Art. R 231-56-6 – This indicates the preventive measures to be taken during **maintenance work** when exposure may be expected to increase considerably. *In the laboratory, this means in particular the maintenance of fume cupboards or replacement of the filters in chemical hoods (ventilated chemical work stations) or laminar flow hoods (microbiological safety cabinets).*

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[6] Decree No. 84-1093 of 7 December 1984 concerning the ventilation and sanitization of places of work.

[7] The Health Code also requires (R 5162) that carcinogenic, mutagenic, teratogenic, toxic and highly toxic products be stored separately from other products. They must be placed in lockable cupboards or premises not freely accessibly to persons from outside the establishment.

The Decree also stipulates:

- that there must be no access to hazard areas for workers other than those whose work or duties require such access (Art. R 231-56-7);
- that suitable training and information must be provided for workers who may be exposed to carcinogenic products (Art. R 231-56-9); and
- that before a worker is assigned to duties which expose him to carcinogenic agents he must first undergo a medical examination, followed by periodic check-ups thereafter, and that his medical record must be kept for 40 years after his exposure ceases (Art. R 231-51-11).

To sum up, the priority imperatives are

- *to assess the risks;*
- *to reduce the use of hazardous substances, replacing them with non-harmful products or, if this is impossible, using carcinogenic products in a sealed environment;*
- *to limit the quantities of products and number of workers exposed;*
- *to demarcate and mark hazard areas, to label all receptacles;*
- *to collect, store and remove waste safely;*
- *to train and inform workers;*
- *to provide medical surveillance.*

2. IDENTIFICATION OF HAZARDOUS SUBSTANCES AND UP-TO-DATE INFORMATION

There are various sources of information on the potential hazards of genotoxic and/or carcinogenic agents. This information is constantly evolving and information on each product used needs to be updated all the time. For this reason the working group preferred not to publish any list claiming to be exhaustive. We can, however, point to a number of sources of information on genotoxic and/or carcinogenic products.

2.1 List of dangerous substances for which a harmonized system of classification and labelling has been agreed at community level Annex I to European Directive 67/548/EEC and its adaptations[8]

This list classifies more than 1000 substances on the basis of the potential danger they constitute. Carcinogens and mutagens are classified in categories 1, 2 and 3 in terms of the degree of certainty that they are human carcinogens or mutagens.

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[8] Latest edition: Official Journal of the European Communities L 258A, Year 36, 16 October 1993, Vol. 1 and 2.

The application of subsection V, summarized above, is mandatory only in respect of the carcinogenic substances classified in categories 1 or 2, or those which are not classified by the EEC but must be treated as belonging to those categories by reason of their properties.

Note: numerous known carcinogens such as aflatoxin B_1, which is a contaminant in food or chemotherapy drugs, are not classified by the EEC's working parties.

Moreover, pending new texts, the lists of carcinogenic substances and processes and the limit values given in the annexes to Labour Ministry circular No. 7-88 of 14 March 1988, superseding that of 14 May 1985 on the prevention of occupational cancers[9], still have legal force.

2.2 Classification of the International Agency for Research on Cancer (IARC)

This centre, an agency of WHO, publishes evaluations of cancer risk which are made by working groups on the basis of published work reporting epidemiological evidence, experimental animal findings or the results of short-term tests.

In 1995, on the basis of more than 790 products, groups of products or industrial processes studied in 57 monographs, the IARC experts identified the following as:

- **human carcinogens (group 1):** agents whose human activity is demonstrated by epidemiological and experimental studies
- 13 exposure situations (industrial processes, social habits and occupational exposures);
- 56 substances or groups of substances or mixtures;
- **probable human carcinogens (group 2A):**
- 52 substances, groups of products or mixtures and 4 exposures;
- **possible human carcinogens (group 2B):**
- 219 substances, groups of products or mixtures and 4 exposures;
- **probably not carcinogenic (group 4):**
- just one substance, caprolactam;
- whilst the others (462) **(group 3)** are considered as **unclassifiable** due to insufficient experimental or epidemiological data).

The IARC's working groups operate a rigorous selection in the substances they study. Major selection criteria are the quantity produced and the number of persons involved in handling or using a compound.

For this reason many of the substances used in our research laboratories have not yet been evaluated.

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[9] See Pluyette: Hygiène et Sécurité, 21st edition, pp. 1656-59, 1993, Lavoisier, Paris.

2.3 Other information sources

2.3.1 Factual databases

- The database which provides information on the largest number of products is the "Registry of toxic effects of chemical substances" (**RTECS**). This is compiled and published by the National Institute for Occupational Safety and Health (NIOSH) and covers 122 000 products. It includes more than 6000 products tested for their tumorigenic effect or presenting a hazard to health as tumorigenic agents, and more than 10 000 files which refer to the mutagenicity of products. This raw information, which is not validated by expert committees, is accessible either on paper or on-line through NLM (TOXNET) or on CD-ROM – CCINFO and CHEM-BANK.

Other TOXNET databases include
- Hazardous Substances Databank (HSDB): contains validated data on more than 4000 products.
- Chemical Carcinogenesis Research Info System (CCRIS): has the results of carcinogenicity studies on some 2500 products.

2.3.2 Databases of bibliographical references

- Chemical Abstracts Service is concerned primarily with chemistry but also includes more than 230 000 toxicology references.
- PASCAL
- TOXLINE
- TOXLIT
- Environmental Mutagen Info Center Backfile (EMICBACK).

2.3.3 Suppliers

It should also be remembered that suppliers are required to indicate in their labelling the potential hazards of products they sell. In catalogues these warning notices feature very differently depending on the supplier,
for example: "Cancer suspected" (JANSEN)
"Cancer suspect agent" (ALDRICH)
"Carcinogenic" or "Warning – possible carcinogen" (SIGMA).

Catalogue information should be treated with caution because it is often incomplete and contains certain inaccuracies in the toxicological data which are best checked from the literature or specialist writings.

Ideally these suppliers should meet their statutory obligations to provide information on hazards by means of informative and legible labelling of receptacles, giving precise information in catalogues and supplying comprehensive safety information sheets as required by law.

Finally, the most stringent rules of safety must be applied to any product on which no information is available, for example during screening studies.

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III.

DESIGN AND ORGANIZATION OF PREMISES

D. MARZIN

DESIGN AND ORGANIZATION OF PREMISES

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Summary

Premises must be fitted out in such a way as to allow clear physical separation between rooms into which mutagenic/carcinogenic products are introduced and those into which they are never introduced, so that any accidental dispersion can be contained.

Working areas must be designed for easy decontamination (walls, floors, ceilings, bench tops). Precautions must be taken to avoid any dispersion during cleaning.

Premises in which carcinogenic products are stored must be identified by a logo (e.g., black crab on a yellow background, see Fig. 1).

The organizational set-up we shall be describing for premises will, of course, need to be adapted to the specific circumstances of laboratories which are already operational.

1. DIFFERENT AREAS OF A DEPARTMENT

1.1 In a department where genotoxic products are handled or stored, there are 5 types of area. In decreasing order of risk these are:

A/ Area into which no genotoxic product may be introduced:
This type of area includes: offices and secretarial workstations, archives, certain microscopic examination rooms, cloakrooms, rest or catering areas. These areas must be clearly separated from the 4 other types by a physical barrier (door, wall).

The rules on safety (see Chapter XII) must stipulate that genotoxic products may neither be stored in nor introduced into such areas, even temporarily (e.g., deliveries or products left on a desk).

Specific case of cloakrooms:
Cloakrooms must be designed in such a way that ordinary street clothing cannot come into contact with work wear, in order to avoid spreading contamination.

B/ Area for storing genotoxic products and weighing materials:
These areas should preferably be located in the same room so that genotoxic products are not moved more than necessary and equipment used in connection with them can be contained within one area.

Storage operations must be conducted under the correct conditions (see Chapter V). These rooms must be kept locked, and access to them is restricted to authorized personnel only; a list of authorized personnel may, for example, be displayed at the entrance to the room.

The weighing area must be properly ventilated (12 air changes/hour and no recirculation). Access to it must be prohibited during handling by means of a warning notice posted outside.

NB: For rooms accessible only to designated personnel, possible options are the keeping of a register of access to the room or a system of entry controlled by magnetic identity card.

C/ Technical handling area:
These include, for example, animal quarters, washing departments, incubator rooms, test rooms and analytical laboratories in which dilute solutions of genotoxic products are used.

D/ Waste storage area:
This room must be kept locked, and access to it is strictly for authorized personnel only; a list of authorized personnel may, for example, be displayed at the entrance to the room.

E/ Preparation area for synthesis and purification of genotoxic products, area in which concentrated solutions, gases or aerosols are used.

1.2 The different types of areas should ideally be kept physically separate from each other. Type C areas must in every case be kept separate from areas of types B, D and E.

The 4 types of area B, C, D and E together with the furniture used in storage and handling must be marked in such a way as to indicate clearly the presence of any genotoxic agents. These areas must be at negative pressure relative to the rest of the building.

During handling of genotoxic products, the doors to these 4 types of area must be kept closed.

These 4 areas must operate at negative pressure relative to the rest of the building so that there can be no escape of genotoxic products.

Ideally areas of type B, D and E in which larger quantities of higher concentrations of genotoxic products are handled should be classed as **"controlled areas"**, accessible only to persons with written authorization; this room should if possible be located in the part of the building which has the least traffic, so that access can be verified and unnecessary movements into and out of it can be avoided.

Before being vented to atmosphere, air from the laboratory should be filtered; in every case, air from hoods or glove boxes in which sizeable quantities of genotoxic products are handled must be filtered.

The recommendations of Harless et al. (1982) are set out in Chapter IV.

Local emergency services must be notified of the existence of hazard areas of types B, D and E.

2. DESIGN AND APPOINTMENT OF THE LABORATORY
(Harless et al., 1982)

2.1 Working areas must be spacious so that work can be carried out in perfect safety.

2.2 Ideally handling areas should have only one point of access into the rest of the building, preferably opening on to an airlock which is glass-walled or has a spy-hole. That is to say, there should be a dividing corridor to act as an "atmospheric buffer zone" in the event of any accidental dispersion.

2.3 Walls and ceilings must be smooth and impermeable, painted with a paint which is resistant to the chemicals and disinfectants routinely used and easily washable (e.g., 3 coats of epoxy-resin paint). Use of false ceilings made of fixed slabs or slats is strictly prohibited, since these encourage the build-up of dust, gases or vapours and are difficult to clean; they also make it easy for products to spread from one area to another.

2.4 Piping and ductwork must be set sufficiently clear of the wall so that dust cannot build up.

2.5 Floor coverings must be non-skid, smooth and easily washable (e.g. heat-welded vinyl sheeting). They should form a rounded angle between floor and wall and continue up to the wall to a height of about 15 cm for ease of cleaning and to prevent the build-up of dust.

2.6 Ideally, laboratories should be visible from the outside through glazed observation windows and doors. Operators can thus be observed by personnel outside and the observer can take the necessary steps if an accident occurs without being contaminated himself. An intercom system is desirable between the handling laboratory and the area outside, avoiding excessively frequent traffic between the two areas.

3. LABORATORY BENCH TOPS AND FURNITURE

Laboratory bench tops must be made of materials which are smooth, impermeable to water, easy to clean, resistant to disinfectants, acids, bases and organic solvents (stainless steel, glass, ceramic glass, corian, large enamelled stoneware slabs or certain plastics may be used). Seam-free bench tops are to be preferred. If seams cannot be avoided they should be tight and easily replaceable, for example silicon seams which must be changed if contamination occurs. Tiled and wooden bench tops are prohibited because they are difficult to decontaminate.

To minimize contamination, handling may be done on absorbent Benchcoat-type paper (which has a plastic backing), placed with the absorbent side facing upwards. This paper must be disposed of together with the contaminated products at the end of each handling operation.

Use of this paper does not mean that the bench top need not be decontaminated after the paper is discarded.

Items of laboratory furniture must be placed in such a way that they are easily accessible for cleaning (e.g., items under bench tops must be mounted on castors); they must have smooth coverings which are easily laundered.

There must be adequate shelving for routine equipment so that bench tops and traffic areas in the laboratory do not get cluttered.

Thoroughfares away from the handling areas must be kept clear of all equipment.

4. CLEANING OF PREMISES AND SURFACES

(Hunt et al., 1982)

The operator is responsible for decontaminating all surfaces (see Chapter XI).

It is recommended that laboratories should be cleaned by a team answerable to the laboratory supervisor; in this way the personnel in this team can be informed and trained and the supervisor will be aware of any staff changes in the team.

Areas likely to be contaminated (defined as areas B, C, D and E in Chapter III) should preferably be cleaned by in-house personnel and in every case by personnel who are given back-up, training and information about the hazards involved.

In order to prevent the generation of aerosols or dust, dry mopping or sweeping must be prohibited. Vacuum cleaners must not be used as they encourage the spread of dust.

Wet cleaning must be used. Sponges are prohibited and must be replaced by disposable materials which are discarded as ordinary waste if they are not contaminated and as mutagenic waste if they are obviously contaminated or it is thought they might be.

In the event of an incident, leak or escape or accident of any kind, or if a receptacle or any other item of equipment topples over or is broken, the safety officer or an experienced laboratory employee must be notified at once.

No attempt should be made to remedy the effects of an incident or accident without the authorization of an experienced member of the laboratory, which means that personnel involved in laboratory studies should receive as much training as possible (see Chapters XI and XII).

No one should enter a room marked "controlled access" without authorization.

The laboratory waste bin should never be emptied unless an instruction to do so has been given or authorization to do so is marked on the receptacle.

5. MARKING AND IDENTIFICATION OF PREMISES

The poster shown in Fig. 1 should be displayed at the entrance to all areas in which genotoxic products are handled or stored.

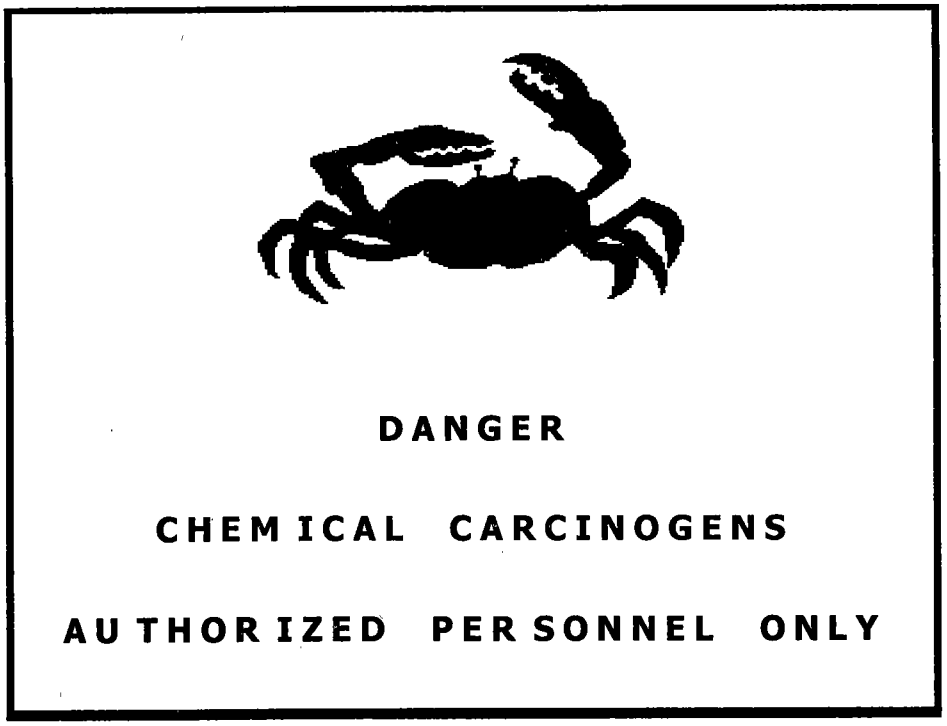

Fig. 1. Poster to indicate areas where genotoxic products are handled or stored

(This poster has not as yet been approved, but is readily identifiable)

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IV.

PROTECTIVE EQUIPMENT AND LABORATORY MATERIALS

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PROTECTIVE EQUIPMENT AND LABORATORY MATERIALS

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Prevention of the hazards entailed in handling genotoxic products is ensured by successive levels of containment which are designed to minimize the dispersion of toxic substances and limit the number of personnel exposed. Overall containment is ensured by the design and organization of the premises (cf. Chapter III).

Hazards to operators and their immediate vicinity are prevented by the use of collective and individual protective equipment and by the choice of appropriate laboratory equipment.

- Collective prevention is primarily a question of ventilation systems. Care must be taken to choose the right equipment for the work being done and its performance must be checked. This equipment must be serviced regularly.

- Complementary to these measures is the wearing of individual protective equipment (personal protection). Some items, such as overalls and gloves, are essential in most handling operations. Others, such as respiratory protection equipment or safety goggles, may be necessary in some handling operations.

- Choice of the most suitable laboratory equipment and proper use of it are also essential preventive measures.

This chapter thus examines, for laboratories which use genotoxic products:

- ventilation systems;
- personal protection equipment;
- laboratory equipment.

It also examines circumstances in which a product is added to a culture medium together with the appropriate equipment to be used in such cases.

1. COLLECTIVE PROTECTION: VENTILATION SYSTEMS

1.1 General ventilation

As far as possible, pollutants emitted in the course of work should be entrained at source by local exhaust ventilation systems. General ventilation does two things: it replaces the laboratory air generally and dilutes and thus reduces the risks from pollutants which are emitted or dispersed in the laboratory's ambient air despite the use of local exhaust ventilation equipment. It also introduces a supply of new air to make up the air removed by the various collective protection systems.

1.2 Local exhaust ventilation

Local exhaust ventilation is the only means of collective protection available to people working in laboratories. It provides the first level of containment mentioned in the introduction, whilst a second level may be provided by the laboratory walls and by setting the laboratory at negative pressure relative to the adjacent premises. Local exhaust ventilation makes use of ventilated enclosures designed to prevent pollutants from escaping into the ambient air of the laboratory. They may be totally or partially enclosed and it will be readily appreciated that the former are intrinsically more efficient than the latter, the quality of which depends on the creation of an inflow of air which prevents pollutants from escaping through the inlet aperture. Pollutants are thus contained within the enclosure and must then be removed from it.

The safest way of evacuating pollutants contained within the enclosure is by venting the polluted air to the atmosphere outside the building where it will be diluted, provided the siting of the premises makes the presence of a discharge flue acceptable and provided a measure of pollution in the outside atmosphere is tolerable; this is the principle of **once-through ventilation**. An alternative principle, **recirculation**, entails separating out the pollutants present in the ventilation air of the enclosure, placing them in a suitable holding system and returning the cleaned air back into the laboratory. From the point of view of clean laboratory air, these two principles are not equivalent. The former is intrinsically safe, but the efficacy of the second requires a separator system suitable for the product(s) being removed, and requires that system to work properly at all times.

1.2.1 Air cleaning

A variety of techniques are used, depending on the nature of the pollutants to be removed: **particles** are removed by **filtering** whilst **gases and vapours** are captured by scrubbers which work by **adsorption** and/or **absorption**.

In the laboratory, solid **particulate** pollutants are collected on very high-performance or absolute filters (commonly termed HEPA or high-efficiency particulate air filters). The technology for these is now well established and their efficacy is defined by US Federal Standard 209DE and French standard NF X 42-070 in particular by reference to the DOP test (whereby one particle breaks through a class EU 14 HEPA filter for every

100 000 particles entering it). The efficiency of filters varies over time. The initial efficiency of filters exposed to solid particulates usually improves as they become clogged, provided clogging remains within the limits set by the manufacturer and provided the pressure drop is offset by good ventilation. In all cases, over-clogging of filters greatly reduces the volume of air treated. ***For these reasons filters must be constantly monitored (measurement of pressure drop around the filter edges) and they must be periodically inspected by counting the proportion of particles which break through.***

Gaseous pollutants are usually collected using activated carbon scrubbers, which must be carefully chosen in relation to the type of pollutants to be collected.

The two criteria for a scrubber are its scrubbing efficiency and its maximum retention capacity which, once reached, impairs scrubbing efficiency. Care must also be taken over the phenomenon of **desorption**, which reduces the efficiency of scrubbers. This can happen when air which is too humid passes through the scrubber or products are present for which the scrubber has a greater affinity than it has for the pollutant being removed. In the latter case, the pollutant originally captured is not cleaned by the scrubber and the second one takes its place.

These reasons for impaired efficiency demonstrate how important it is to check that scrubbers are working properly. Inspection may be continuous or intermittent. The former method uses a detector which is only suitable for certain pollutants. Intermittent methods involve testing downstream of the scrubber using a sampler tube or other suitable method. The drawback with these is that they cannot pinpoint the moment at which the scrubber begins to malfunction. Inspections must be all the more frequent, the closer the scrubber gets to its maximum retention capacity.

These factors in the recirculation of laboratory air clearly demonstrate that the technology involved should only be entrusted to specialists properly qualified in the choice and maintenance of filters and scrubbers; one cannot overstate the importance for users of obtaining every possible guarantee. In the absence of these guarantees, they should <u>not</u> recycle their laboratory air.

Laboratories fitted with "once-through" direct venting systems which extract their ventilation air need to be supplied with a flow of new "make-up" air equal to the total volume extracted. This general make-up air must preferably be supplied by mechanical means if one is to have reasonable control over the quality and distribution of the make-up air. This is a principle which is often neglected, and failure to observe it can seriously impair the working and efficiency of systems which vent to atmosphere. Another advantage of mechanical air replacement is that it allows the pressure of the laboratory to be set at a given level relative to the volumes around it. Thus, a laboratory which needs to be protected against pollutants present in the environment outside can be set at slightly positive pressure, whilst conversely if one wishes to protect adjacent

premises against pollutants present inside the laboratory, the laboratory can be set at slightly negative pressure. Positive pressure must be prohibited in laboratories which handle genotoxic substances. The following installation rules must also be observed:

- for each ventilated hood served in the laboratory there must be ductwork and an extractor which has a back baffle installed;
- there must be no possibility of air vented via the building's general ventilation systems re-entering the building (e.g. because vents are too close to the building or prevailing winds blow in a certain direction).

*1.2.2 **Legislation** (INRS, 1991)*

The underlying principles of the legislation on ventilation and sanitization of places of work (Decrees 84-1093 and 84-1094 of 7 December 1984) are as follows:

- objectives and obligations must be built in to ventilation systems from the design stage;
- the efficiency of ventilation equipment must be checked when it first comes into service;
- this equipment must be periodically inspected and a record kept of the system;
- stricter obligations exist in respect of air recirculation systems.

*1.2.3 **Ventilated laboratory enclosures** (Cornu & Gaillardin, 1993a,b)*

It is unfortunate that there is no standard terminology for ventilated laboratory enclosures. The use of different terms for one and the same item of equipment or, conversely, of one term to designate different items of equipment, does not make for ease of understanding between interlocutors and, more seriously, it can lead to mistakes when decisions are taken as to when and where such enclosures should be used. The terminology employed here is based where possible on definitions given in the standards (fume cupboard, microbiological safety cabinet, laminar flow dust-free work station). Alternatively, it is terminology which we recommend as a way of avoiding terms whose significance has become blurred by usage (hoods, laminar flow). The purpose of Table 1 is to enable the reader to cross-check between common terms and the terms we recommend.

1.2.3.1 Fume cupboards (AFNOR, 1987a,b)

These are ventilated hoods at negative pressure which draw air into the laboratory and vent it to atmosphere outside the building by means of a fan. Because the air drawn into the laboratory is not cleaned before it enters the working zone of the fume cupboard, **all these can do is protect personnel against pollutants liberated inside the fume cupboard (particulates or gases) and any moderate splashes or explosions which may occur there.**

Table 1. Terminology for ventilated laboratory enclosures

Common terms	**Standard terms or those recommended to avoid confusion**
Hood	Fume cupboard (NF X 15-023) Ventilated chemical work station Microbiological safety cabinet (NF X 44-201) Laminar flow dust-free work station (NF X 44-102)
"Biohazard" sterile hood	Microbiological safety cabinet Class II (NF X 44-201) Laminar flow dust-free work station (NF X 44-102)
Laminar flow hood	Microbiological safety cabinet Class II (NF X 44-201) Laminar flow dust-free work station (NF X 44-102)
Glove box	Scrubber glove box Microbiological safety cabinet Class III (NF X 44-201)
Horizontal laminar flow hood	Laminar flow dust-free work station (NF X 44-102) Two types: horizontal inflow and Horizontal outflow
Vertical laminar flow hood	Laminar flow dust-free work station (NF X 44-102) Three types: vertical flow without Reintroduction; Vertical flow with total Reintroduction, or vertical flow with partial reintroduction
Portable hood Mobile hood Chemical hood	Ventilated chemical work station

Protection against pollutants is provided by an inflow of air from the inlet aperture to the working zone. If it is sufficiently powerful, homogeneous over space and stable over time, this airflow keeps the pollutants contained and vents them to the atmosphere outside the building.

Protection against splashes is provided by placing a mobile screen between the work and the operator. Depending on whether the need is to protect the operator's face or whole body, the screen may be either:

- a single panel, extending vertically and enclosed, with an opening just big enough to admit the forearms;
- several panels, extending horizontally, arranged in such a way that the operator can work but still be protected.

There is also a combination whereby horizontally extending panels are set in a vertically extendible screen.

Air velocities through the screen aperture must be adequate but remain moderate if toxic substances are to be effectively contained. An average air velocity of 0.4 to 0.6 m/sec appears to be the most widely accepted value.

Air currents caused by open doors and windows, make-up air blowers or operator traffic close to fume cupboards can seriously perturb the stability of the airflow and impair containment (Cornu & Gaillardin, 1993c). For this reason, a fundamental parameter of the efficiency of fume cupboards is not only their design or manufacture but also the

place where they are sited in the laboratory, and careful consideration must be given to this. The user too has an important part to play in protecting himself, by opening the screen as little as possible, not cluttering the fume cupboard unnecessarily or blocking the extractor vents and by generating any pollutants as deep as possible inside the fume cupboard.

1.2.3.2 Microbiological safety cabinets (AFNOR, 1984)

These are not intended for use in the handling of toxic, and particularly genotoxic, products except in the specific cases described later by Simons & Sotty (1991).

There are three types which differ from each other in the technology they use and the levels of protection they afford. In terms specifically of the objectives of protection they purport to meet:

- cabinets of **class I** protect **personnel and the environment**;
- cabinets of **class II** protect **personnel and the atmosphere in the laboratory and around the product** against both pollutants from the laboratory and from other products being handled at the same time inside the cabinet (cross-contamination);
- cabinets of **class III** are designed more particularly for the handling of biological agents of class 3 or 4. These protect **personnel and the environment against hazardous agents handled in the cabinet and protect the product handled against pollutants from the laboratory.** However, the degree of protection for personnel is higher than that afforded by cabinets of types I and II because, unlike them, types III have a physical barrier between the hazardous agent handled and the laboratory atmosphere.

It is mandatory for cabinets of types I and III to have a direct connection venting them to atmosphere. Note: cabinets of class III which claim to be "convertible into class I" should be avoided as the "conversion" may lead to problems.

The most popular version of class II cabinets recycles air which has been removed from the laboratory back into it. It should be borne in mind that personnel and the laboratory atmosphere are then only protected if two conditions are simultaneously and permanently met:

- efficient containment of pollutants in the cabinet's working zone, which presupposes that there is an airflow entering from the inlet aperture which is powerful and stable, and that there are no perturbing air currents;
- HEPA filtering of air blown out of the cabinet into the laboratory atmosphere.

Since the end of 1994, cabinets of class II have undergone a procedure of certification of compliance with the French standards (NF). Purchasers of equipment which complies with the standard have a guarantee that it meets all the specifications of NF X 44-201 plus the additional specifications of the certification scheme.

1.2.3.3 Laminar flow dust-free work stations (AFNOR, 1983)

Depending on the velocity and direction of the airflow in the working zone one may have:

- **horizontal inflow** hoods that **do not protect the product**, which is bathed by the laboratory air, and **do not protect personnel either** because the force of the air inflow through a large aperture is low;
- **horizontal outflow** hoods that **protect the product** by feeding a supply of dust-free air to the working zone but **expose the operator** because there is a direct outflow of air which may be polluted;
- **vertical flow hoods without reintroduction**, which are **equivalent to horizontal outflow hoods**;
- **vertical flow hoods with total reintroduction or partial reintroduction,** which **protect the product** but **offer no guaranteed protection for personnel.**

Ultimately, the only practical value of laminar flow dust-free hoods is that they guarantee a working zone which is protected against outside pollutants. **They are <u>not</u> a means of collective protection for personnel.**

1.2.3.4 Ventilated chemical work stations

The design of these is in many respects similar to that of class I microbiological safety cabinets, with the difference that the latter are fitted with HEPA filters whilst ventilated chemical work stations have an activated carbon filter to entrap gases. **The scrubber must be chosen and maintained in line with the requirements set out in section 1.1.**

1.2.3.5 Scrubber glove boxes

These are enclosures similar to class III microbiological safety cabinets, with the difference that their outlet is fitted with a gas scrubber. **The scrubber must be chosen and maintained in line with the requirements set out in section 1.1.**

1.2.4 Local collectors (mobile or fixed)

There is a wide range of equipment, from "directable nozzle" collectors to mobile suction hoods or fixed collectors adapted for use with a specific apparatus or type of work. In the case of fixed collectors adapted by professionals for use with a specific apparatus, it is enough to ensure that they are switched on when the apparatus is in use. As regards the other systems, it should be borne in mind that they are only effective when very close (sometimes 15 cm or closer) to the point where the pollutant is emitted (flask, cup, trough) and provided there is no counter-current of air... As a general rule, these collectors protect considerably less well than ventilated enclosures and are thus not recommended for the handling of genotoxic substances.

1.2.5 Choice of ventilated laboratory enclosures

The choice is a difficult one. It should take into consideration parameters such as the following:

- the protection objectives required (protection of personnel and /or the product and/or the environment);
- features of the pollutants against which protection is required (physical properties, toxicity, quantities handled);
- whether or not suitable filters or scrubbers are available;
- constraints on installing and using certain types of equipment;
- inspection facilities available.

In view of the above and the fact that the handling of genotoxic materials is likely to lead to the simultaneous production of both solid- and gaseous-phase pollutants, the **laboratory fume cupboard**, perhaps combined with filtering and/or scrubbing of the waste air, would seem to be **the method of choice for collective protection.**

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2. PERSONAL PROTECTION EQUIPMENT

There are two European directives concerning personal protection equipment:

- Directive 89/656/EEC of 30 November 1989 lays down minimum requirements for workers' **use** of personal protective equipment. It points to the paramount importance of putting in place measures for collective protection and good working methods. It sets out employers' obligations with regard to assessing risk, selecting the most appropriate personal protection equipment, maintaining this equipment and issuing it to workers, and informing and training workers in the correct wearing and use of personal protection equipment.

- Directive 89/686/EEC of 21 December 1989 lays down conditions for the **design**, marketing and free movement of personal protection equipment within the EEC. It specifically stipulates essential requirements in regard to health and safety which this equipment must meet, with principal reference to efficiency, comfort and ergonomic design, and lack of harmful effects for the user. Moreover, it stipulates the certification procedures which such equipment must undergo, together with the content of the notification which the manufacturer must draw up for each item of equipment.

A number of legislative texts have incorporated these directives into French law. There are also harmonized European standards. Others are being prepared. If equipment conforms to these standards it may be deemed to meet the essential requirements of the directive.

2.1 Protective clothing

In all areas where genotoxic products are used protective clothing must be worn, chosen on the basis of the nature of the product used and the level of hazard.

Overalls may be of woven or non-woven fabric. During use they must not leave the premises. Overalls of different colours may be chosen as a reminder that they are not to be worn outside the laboratory where genotoxic products are handled.

In normal use, overalls must be changed periodically. They must be discarded immediately when obviously contaminated. Overalls for disposal must be packed in plastic sacks, correctly labelled as mutagenic or carcinogenic, and placed in special bins for subsequent incineration.

2.2 Gloves

2.2.1 Choice of gloves

Resistance of gloves to chemicals and microorganisms is covered in the 1994 French standard NF EN 374. Chemical resistance *per se* is evaluated in terms of leak tightness and resistance to permeation.

A tightness rating of 1 to 3 is given based on the result of a test of penetration by air or water defined in standard NF EN 374-2, the choice of test method depending on the material. It should be pointed out that resistance to microorganisms is judged purely by the hypothesis that gloves resistant to penetration offer enough of a barrier to microbiological hazards.

This classification is based on a level of manufacturing quality. It offers no absolute guarantee of tightness. For protection against genotoxic products it is imperative to choose gloves with the rating 3 which offers the best probability of tightness.

Resistance to permeation is determined by a specific test method which evaluates the behaviour of a material used in glove manufacture when it is exposed to a specific chemical product.

A rating of 1 to 6 is given depending on the result of a permeation test using the method specified by standard NF EN 374-3. Extrapolating from the result for one product to another product offers no guarantee of protection, even if the two products are from the same chemical family.

In choosing a glove one must consider the genotoxic chemical itself but also the solvent, which may have a more powerful effect on the membrane constituted by the glove. Aflatoxins, for example, may be present in solution either in chloroform or in dimethylsulphoxide (DMSO).

It should be borne in mind that the chemical resistance of a protective membrane to a given chemical product is a specific property. Thus there is no single type of glove suitable for all products handled in the laboratory. Furthermore, chemical resistance may change during use as a result of chemical degradation of the glove material or as a result of slow penetration of that material by the chemical product. Thus there are no gloves which give permanent protection. Consequently, gloves must be chosen carefully with a view to the products handled and they must also be changed at frequent intervals.

The choice of gloves is necessarily a compromise between different and conflicting characteristics: chemical resistance, mechanical resistance and ergonomic characteristics.

Work in the laboratory requires considerable dexterity. Lightweight gloves for single use are usually used. These single-use gloves are made primarily of natural latex, vinyl (PVC), nitrile (butadiene-acrylonitrile copolymer). These materials do not offer satisfactory resistance to all chemicals.

Disinfectant treatments may weaken glove performance. A Swedish study has shown that gloves of latex, PVC and polythene no longer provide adequate protection after treatment using ethyl alcohol or isopropyl alcohol. They are not affected, however, by treatment in solutions of glutaraldehyde or para-chlorometacresol.

Protective creams, sometimes wrongly termed "liquid gloves" are no substitute for personal protection equipment. Circular 89/6 of 2 February 1989 indicates that

protective creams must not be used in direct contact with preparations which are particularly hazardous on contact with the skin. Use of these creams when handling genotoxic products should thus be prohibited.

2.2.2 Rules for use

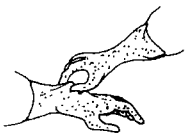
Gloves offer protection for a limited period of time only.

> Chemicals can migrate through the membrane which the glove constitutes (permeation). Gloves may also suffer chemical or mechanical degradation.

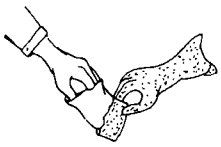
They must thus be changed at frequent intervals.

Single-use gloves must be discarded immediately following contact with carcinogenic products. When removing gloves, care should be taken not to soil the exposed hand.

To remove gloves soiled by a toxic chemical proceed as for gloves contaminated by radioactivity, as shown in the drawings below. Store them afterwards as solid toxic waste.



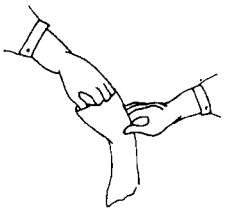
1. Take hold of glove a few cm below the wrist



2. Turn inside out, pulling until the fingers emerge

3. Using fingers still protected by the inside-out glove, pull off other glove

4. After turning second glove fully inside out place in toxic waste receptacle



5. Complete removal of first glove.

Drawing H. Pastor - INSERM

In some cases the risk of exposure can be significantly reduced if two pairs of gloves are worn one on top of the other, either of the same or of a different kind. This method may be used provided it allows the wearer enough sensitivity of touch. Another benefit of this method is that one can change gloves and at the same time remain protected. When the outside pair becomes contaminated it is removed so as not to contaminate the laboratory equipment used and all other objects handled (doorknobs, etc.).

2.3 Respiratory protection equipment

2.3.1 Types

There are many types of respiratory protection equipment. A first distinction has to be made between respirators, which incorporate a filter, and breathing apparatus.

Filter respirators, which clean contaminated ambient air, consist of a facepiece which to a greater or lesser degree covers the airways (nose and mouth) and incorporates a filter. In some cases the facepiece is itself a filter. The respirator may filter out dusts and aerosols, gases and vapours or all these types of pollutants.

Breathing apparatus supplies breathable air from a non-contaminated source and enables the user to be independent of the surrounding atmosphere. These types consist of a facepiece and an air supply. The air source may be carried by the user together with the apparatus. In this case it is a self-contained breathing apparatus. Alternatively, the user may be connected to a source of compressed air which delivers (fresh) air from a non-contaminated area to him through a hose.

In the laboratory, breathing apparatus, whether self-contained or not, is used only in exceptional circumstances, for example to equip the rescue and emergency team in the event of accidental contamination by a highly dangerous substance.

In most cases, filter respirators are used when the features of ventilation equipment are not adequate to guarantee clean air or when work giving rise to gases, vapours, mists, aerosols, etc. cannot be performed under an extractor hood.

2.3.2 Filters

There are filters for aerosols, filters for gases and combined filters. Performance categories exist for aerosol filters (P1, P2 and P3). These performance categories should not be confused with the categories used to rate containment levels and associated apparatus in a biology laboratory.

Similarly, there are categories of protection for gas filters, based on their trapping efficiency. There are also different types of gas filters depending on the nature of the gases or vapours they are required to filter.

Saturation time is the parameter which determines the actual protection time of a gas filter in use.

2.3.3 Choice of respiratory protection

When choosing respiratory protection equipment, account should be taken of:
- oxygen levels;
- the nature of pollutants (gases, vapours, dust), and their ambient concentrations;

- the toxicity of these pollutants (threshold limit values if any);
- particle size in the case of aerosols;
- temperature and humidity conditions;
- the level of physical exertion required of the user;
- the duration of the work being done.

2.3.4 Use

Respiratory protection must be worn and used in accordance with the instructions for use provided by the equipment supplier. The ability of an individual to wear this equipment, which involves a number of constraints, should be assessed, if necessary by consulting a doctor.

Facepieces should be checked to ensure proper fit.

Anyone using respiratory protection for the first time must be given theoretical and practical training by a competent person.

Periodic training or refresher courses are needed by everyone who uses respiratory protection regularly.

2.4 Safety goggles

The wearing of goggles is essential where there is a risk of splashes.

These goggles must have protective sidepieces.

Where there is exposure to irritant vapours, protection may be provided by a respirator or breathing apparatus, the facepiece of which is a full-face mask covering eyes, nose, mouth and chin, and which incorporates a visor.

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3. LABORATORY EQUIPMENT

3.1 Pipetting equipment

It is strictly prohibited to pipette by mouth irrespective of the nature of the solution being transferred.

There are a number of pipetting systems. For example, a high-performance "pipet-aid" system which allows the desired volumes to be accurately transferred and distributed. All types of pipette including Pasteur pipettes can be adapted to this. The use of rubber pipette fillers should be prohibited. Whatever the equipment chosen, pipetting should be done gently to prevent the formation of mini-aerosols, splashes or small droplets.

For distributing small volumes there are automatic distribution systems, of the micro-pipette type, with disposal tips. Only the tips are in contact with the hazardous solution. Prior to handling, a receptacle should be placed in readiness to collect used tips, so that they are not thrown into waste bins. Once sealed, these receptacles should be removed together with mutagenic waste by a specialist company.

When transferring mutagenic or carcinogenic solutions preference should be given to single-use pipettes. Where re-usable pipettes have been used, they must be decontaminated by the user before being sent to the washing department.

It is essential to use receptacles made of indestructible plastic (e.g. "Medinette") to store contaminated pipettes prior to disposal, so that there is no risk of the plastic bags being pierced. These leak-proof containers are specially designed to prevent any escape of

contaminated products. They are destroyed in an incinerator by the specialist waste disposal company.

3.2 Other laboratory equipment

- CO_2 incubator, if possible ventilated, with aspiration and removal of air after suitable filtering.

- Bacteriology incubator: ideally this should have an air supply system whereby the air is always filtered before the cupboard is opened.

- Weighing balance: this should preferably be located in an isolated room which is quiet and draught-free.

- Ventilated helmets for weighing.

- Sink, preferably of stainless steel or tough plastic.

- Troughs for carrying flasks from the weighing point to the laboratory where they are to be handled. These troughs must be made of transparent polycarbonate (avoid glass troughs which are too fragile) and must be closed and held shut by clips. If contaminated these troughs must be discarded with mutagenic waste and removed by a specialist company.

- Drying agent granules, e.g., vermiculite. Carrying troughs must contain an adequate volume of granules capable of absorbing a volume equal to that of the granules themselves.

- Inside carrying troughs, tubes and bottles or flasks must be placed in a stable rack. There are also stainless steel carrying troughs which can be decontaminated in the event of an accident. These must be inspected under a safety hood because they have the drawback of not being transparent.

- Airtight screw-top bottles, flasks and tubes with Teflon caps.

- "Benchcoat"-type absorbent paper used with the non-absorbent side facing downwards. **Wherever the method permits, the use of hermetically sealed bottles and flasks is recommended.**

- Leak-proof flasks to hold waste solutions which are mutagenic or carcinogenic. The flask material must be compatible with the solvent used.

- Disposal dishes and glassware to be discarded after use in heavy-duty, perforation-resistant bags of kraft paper lined with polyethylene, for disposal in the hazardous waste drums.

- Disposal sterilizing filter units suitable for use with solvents, of the "Nagène" type, 0.22 μm or 0.44 μm, to be adapted between syringe and needle. These filter units are essential, for example, when handling cell cultures if the cells are to be treated with solutions of mutagenic products, since these solutions must be sterile. Used filters must be placed in heavy-duty bags and transferred to the hazardous waste disposal drums.

- Sterile bacteriological Petri dishes, with vents, for the Ames assay.

- Petri dishes for cell cultures.

- Screw-top dishes for cell cultures. This type of dish should be used where cells are treated with volatile mutagenic or carcinogenic solutions (e.g. dimethylnitrosamine).

- Where such solutions are handled, the dishes containing cell cultures treated with mutagenic volatiles should be incubated in a ventilated cupboard fitted with an extractor system.

- Centrifuges with independent rotors, aerosol-free, which can be decontaminated if a flask breaks. In no event should tubes placed in the centrifuge be covered with parafilm.

4. SPECIAL CASE OF ADDING CARCINOGENS TO CELL OR BACTERIAL CULTURES

Genotoxic substances are often used in experiments, being added to bacterial or cell cultures. In such cases it is necessary to have equipment which makes for good culture conditions but also allows genotoxic substances to be added without exposing the operator unnecessarily. "Laminar flow" equipment is the type most frequently used in these cases. Before going further, it would seem expedient to make a number of useful points in the preamble which follows.

4.1 Preamble

We shall be dealing here only with the handling of ready-prepared solutions of genotoxic molecules. The use of laminar flow equipment is justified only by the presence of cultures, whether bacterial or cell. However, equipment of this type, and more precisely certain types of microbiological safety cabinets, are widely described in many articles as suitable for the **preparation** of solutions of genotoxic substances and providing protection primarily for the operator and the environment. But there are many reasons to question the true efficacy of this equipment in protecting operators during the preparation of solutions of genotoxic substances. The most important of these reasons are as follows:

- microbiological safety cabinets are designed to protect the operator only against exposure to infectious aerosols;

- the fitting, as a precaution, of an additional activated carbon filter has the effect of reducing the performance of the fans which are part of this equipment. The protective performance of these machines is thus likely to be seriously impaired;
- the presence of an activated carbon filter may, if the solvents used are of varying kinds, also lead to the genotoxic substance being desorbed and re-entering the work atmosphere by simple diffusion. Where water is the only solvent used, frequent use of the activated carbon filter may cause it to disintegrate.

The draft European standard currently being prepared and entitled "Performance criteria for microbiological safety cabinets" states clearly in its introduction that these cabinets are not designed for the handling of radioactive, chemical or genotoxic products.

We think it appropriate to end this preamble by emphasizing that:

Microbiological safety cabinets and, by extension, "vertical laminar flow" hoods, **are not designed** to guarantee the protection of operators during the handling and preparation of solutions of genotoxic substances.

4.2 Different types of laminar flow equipment

Depending on the characteristics of cultures, bacterial or cell, the various types of equipment which may be used fall into one of three main categories:

- Horizontal laminar flow hoods
- Vertical laminar flow hoods
- Microbiological safety cabinets

Microbiological safety cabinets are of three types (classes I, II and III). Not all of them are laminar flow equipment.

4.3 Choice of equipment

Bacterial or cell cultures are prepared under different types of laminar flow equipment. Depending on its category, this equipment provides a varying degree of protection to the work, the environment and the operator.

The addition of solutions of genotoxic substances restricts the choice of equipment types which may be used.

4.3.1 Horizontal laminar flow hoods

These were the first models to be used in biology, mainly for cell cultures. This equipment protects **only** the work. Its operating features are shown as a schematic diagram in Fig. 2.

It MUST NOT BE USED when solutions of genotoxic substances are added to cultures.

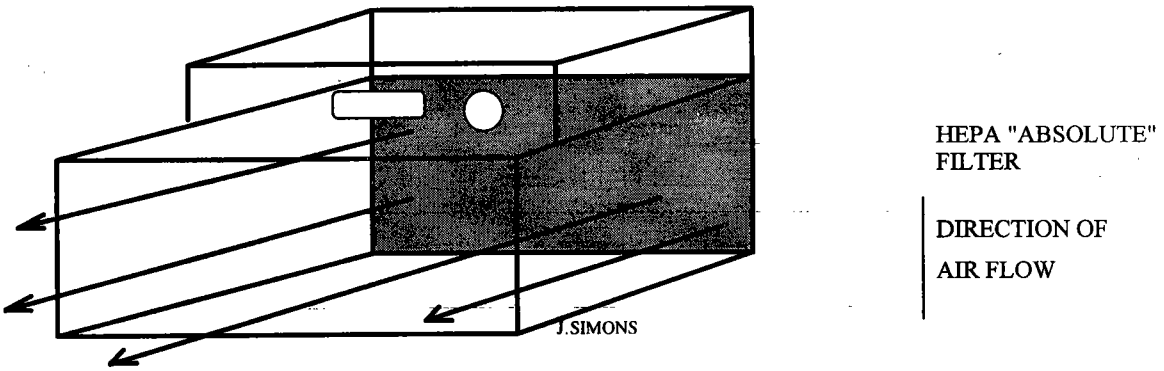


Fig. 2. Schematic diagram showing operation of a horizontal laminar flow hood

There are also horizontal laminar flow hoods with an exhaust action. These protect **NEITHER** the operator **NOR** the work. Their use should also be **prohibited.**

4.3.2 Vertical laminar flow hoods

Vertical laminar flow hoods may recycle all or part of the air. The air, if extracted, may or may not be filtered and the filters used may vary in quality up to the quality of a HEPA filter. Fig. 3 shows the operation of the model with partial recycling, which is the most widespread.

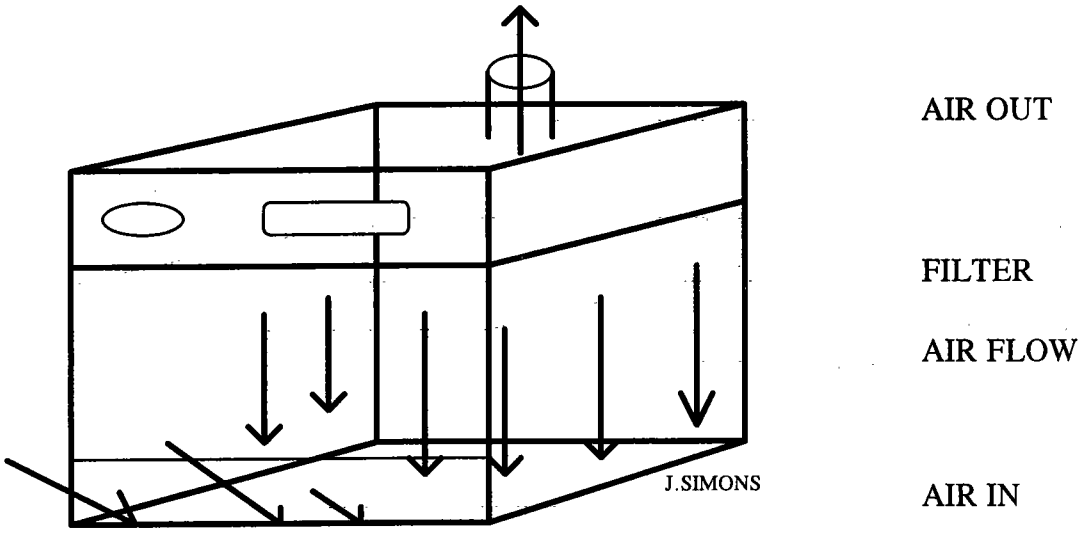


Fig. 3. Schematic diagram showing operation of a vertical laminar flow hood

Vertical laminar flow hoods vary greatly in terms of their fittings and accessories. They provide very poor protection for the operator, the environment and the work. Like horizontal laminar flow hoods, these hoods are not covered in France by any **standard which stipulates their characteristics, protective performance and methods for assessing them.** The only standard which refers to them is NF X 44-101. The purpose of this is to define dust levels at indoor workplaces.

Vertical laminar flow hoods are thus NOT RECOMMENDED where genotoxicants are added to cultures.

4.3.3 Microbiological safety cabinets

All these cabinets provide simultaneous protection for the operator and the environment and, depending on their class, the work. Classes II and III protect all three, whilst class I does not protect the work. Fig. 4 is a schematic diagram of a class II microbiological safety cabinet. These models are the most widespread since they are not subject to the technical constraints of cabinets of types I and III, for example a connection venting directly to atmosphere.

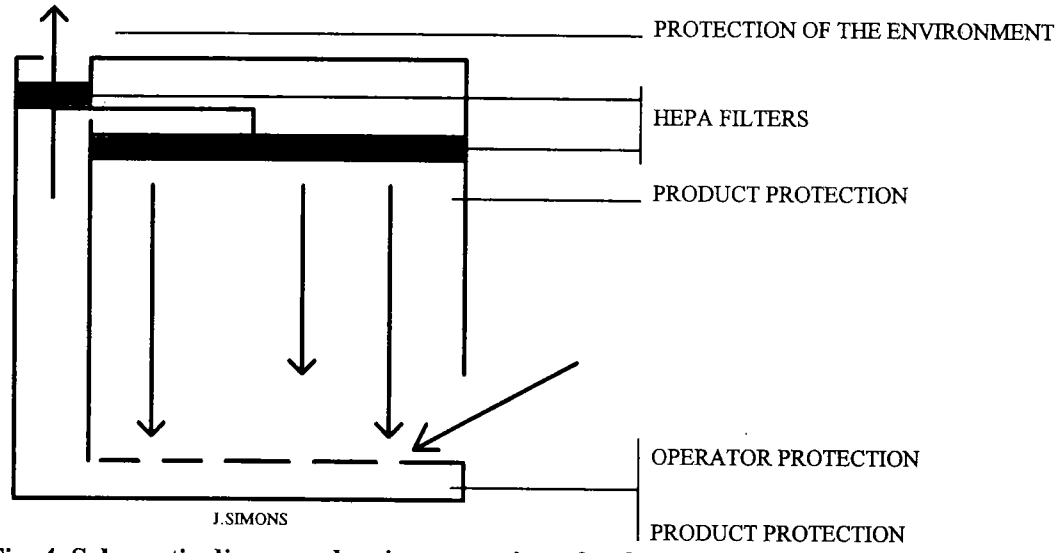
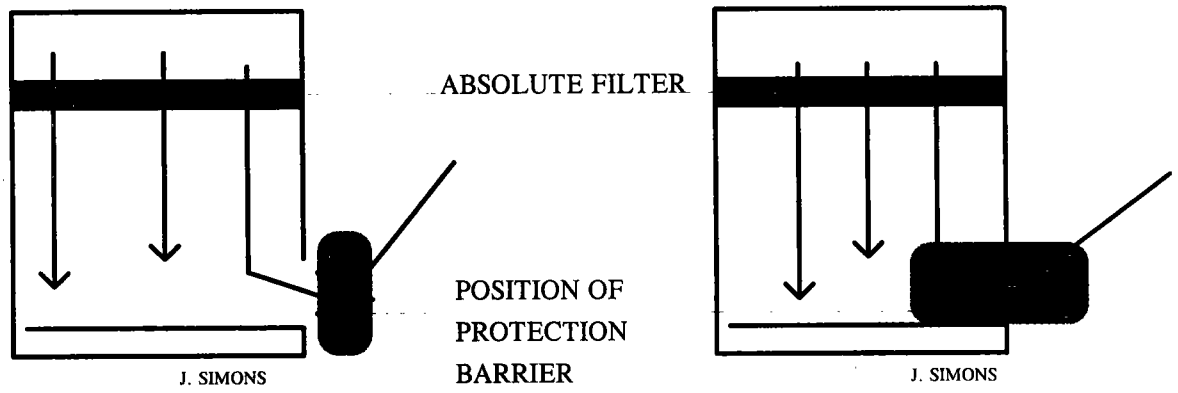


Fig. 4. Schematic diagram showing operation of a class II microbiological safety cabinet

Microbiological safety cabinets of class II differ from vertical laminar flow hoods by virtue of their design. The effect of the design is that simultaneous protection is offered to the operator and the work by the position of the protective sash which is explained in Fig. 5.

There is in France a specific standard on microbiological safety cabinets (NF X 44-201) which regulates the names by which they may be known, their design, their performance and inspection methods for them.



VERTICAL LAMINAR FLOW HOOD

CLASS II MICROBIOLOGICAL SAFETY CABINET

Fig. 5. Respective positions of the protective sash in a vertical laminar flow hood and a class II microbiological safety cabinet

When genotoxic solutions are added to cell or bacterial cultures, this must be done under a microbiological safety cabinet, even though these cabinets are not designed for this application.

The choice of class of microbiological safety cabinet will be based on the criteria below, which are not exhaustive. A further criterion, where appropriate, is the wearing of personal protection.

4.3.3.1 Class I microbiological safety cabinets

These protect the operator and the environment. **They must conform to NF X 44-201.** If the room is at negative pressure the necessary connection which vents the cabinet to atmosphere may compromise the negative pressure.

4.3.3.2 Class II microbiological safety cabinets

These protect the operator, the work and the environment. In France these carry a **quality mark** which provides the purchaser with a guarantee of manufacturing quality and proven performance. In all cases they must, at least, **meet the standard NF X 44-201.** When adding genotoxic substances to cell or bacterial cultures, the operator can protect himself fully by wearing a mask and safety goggles. The cabinet can, if required, be connected directly to a discharge flue provided care is taken to ensure that this will not impair its performance.

Note: never fit an additional activated carbon filter.

4.3.3.3 Class III microbiological safety cabinets -

These protect the operator, the work and the environment. **They must meet the requirements of NF X 44-201.** These would appear to be the equipment of choice where solutions of genotoxicants are added to cell or bacterial cultures. However, they do require direct venting to atmosphere, which restricts the sites at which they can be used, as in the case of cabinets of class I. Replacement of the gloves with which they are fitted can also expose operators to contact with genotoxic molecules. In all cases, handling under a class III cabinet requires the operator to have some experience of working in confined spaces. The constraints this imposes are felt by many to be a nuisance.

Note: these cabinets must not be converted into cabinets of class I.

To sum up, the addition of solutions of genotoxicants to bacterial or cell cultures must take place under a microbiological safety cabinet, with due awareness of the fact that this equipment is not originally designed for these applications. The choice of class of microbiological safety cabinet should be dictated by the operator's objectives and his experience of working in confined spaces.

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V.

ACCEPTING, RECORDING, CONDITIONING, STORING & TRANSPORTING GENOTOXIC, MUTAGENIC AND/OR CARCINOGENIC PRODUCTS

Y. COURTOIS, M. CASTEGNARO, C. BEAUBESTRE

ACCEPTING, RECORDING, CONDITIONING, STORING AND TRANSPORTING GENOTOXIC, MUTAGENIC AND/OR CARCINOGENIC PRODUCTS

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Chemicals which are genotoxic, mutagenic and/or carcinogenic must, whether in their initial form (solid, liquid or gas) or in solution (concentrated or diluted), be stored in locked cupboards (NB: certain products have to be kept refrigerated: in this case flameproof refrigerators or ice chests must be used, fitted with an auditory/visual alarm system) and kept separate from all other substances (Article R 5162 of the Public Health Code) in specially appointed areas which are properly ventilated and monitored at all times. Storage areas must be marked "**DANGER – CHEMICAL CARCINOGENS – AUTHORIZED PERSONNEL ONLY**" and must be marked with the special logo for carcinogenic products. Receptacles in which genotoxic, mutagenic and/or carcinogenic products are transported and stored must be made of a material which cannot be attacked by the solvent or the product itself. Since 1 January 1995 the transportation of genotoxic, mutagenic and/or carcinogenic products has been governed by the Order of 12 December 1994 on rules for the carriage of hazardous materials (rules on carriage by road and alphabetical classification of materials), published in the *Journal Officiel de la République Française* of 27 December 1994 and the *Recueil de Documents Administratifs* No. 113 of the same date.

1. SUPPLY AND PRIMARY STORAGE

Stocks of carcinogenic products must be managed in a way which avoids unnecessary dispersion of them; they must be kept as close as possible to the point of use to keep transportation of them to a minimum.

In every establishment there must be an official responsible for ordering, recording and overseeing the stocks. He must ensure that the product is not already available within the department or another department of the establishment and must order only the minimum quantities needed for the proposed experiments (or the minimum quantity available commercially). He is told when the product arrives and accepts delivery of it. To avoid later problems with weighing, products can be ordered ready-weighed and conditioned (e.g. Isopac). If that is not possible, pre-conditioning is recommended as soon as the product reaches the laboratory (cf. Chapter V).

Each product should be numbered (for easier retrieval) and recorded in an inventory (register or file) which must state:

- its common name, chemical name and formula, commercial origin, CAS number;

- physical, chemical and toxicological properties;
- date of delivery, quantity delivered;
- storage, conditioning and disposal methods (for details of these methods see Chapters IX and X);
- quantities drawn from stock, when drawn (date) and by whom;
- number and location of product.

Sample record of use sheets are given below.

Product name : **CAS No :**

RECORD OF USE SHEET

Date	Quantity Drawn	Person drawing	Solvent	Concentration	Solution volume

Name of product : **CAS No. :**

N° of product : **Location :** **Shelf No. :**
Tray No.:

Other names :

Chemical formulae :
Crude **Developed** **Molecular weight**

Symbol / Risk phrase and warning :

Commercial provenance : **Batch :**

Date received : **By :**

Quantity received :

Conditioning changed on : **By :**

Type of original conditioning :

Type of new conditioning :

Form : solid ❒
liquid ❒
solution ❒ **Solvent :**

<u>Physicochemical properties :</u>

Purity : **%**

Density :

Solubility :

Storage conditions :

References for methods of disposal/decontamination :

<u>Toxicological properties :</u>

Carcinogenic ❒ **no** ❒ **yes** ❒ **suspected** ❒ **unknown**
Mutagenic/ Genotoxic ❒ **no** ❒ **yes** ❒ **suspected**
Other known toxic effects : ❒ **unknown**

I.A.R.C. / C.I.R.C. Classification :

E.C. Classification :

1.1 Solid or liquid products

Following acceptance and recording, products are stored in their original bottles in unbreakable containers which are resistant to the products concerned (metal or polycarbonate receptacles may be used which can easily be chemically decontaminated in the event of an accident). Containers must be hermetically sealed and must not open if dropped; bottles are packed inside the containers in a material which will absorb liquids and which cushions against breakage if the container is knocked or jolted.

Genotoxic, mutagenic and /or carcinogenic products must be stored in a locked cupboard (ventilated cupboard whose exhaust air is filtered, refrigerator, cold room), placed in a properly ventilated room to which there is no free access for persons from outside the department. This area is classified as a "**HAZARD AREA**". The key to the cupboard is held by the supervisor who surrenders it to authorized persons only and ensures that the record of use sheet has been properly filled out. Specialist signs giving the appropriate information alert users or persons from outside the laboratory to the hazards involved. Local emergency services must be notified of the existence of **HAZARD AREAS** (cf. Chapter III).

1.2 Gaseous and highly volatile products

These products must only be used in laboratories specially equipped to handle toxic gases.

Following delivery and recording, products are stored in their original conditioning in a special room which is properly ventilated and equipped with an automatic extractor system with appropriate gas collection apparatus. Electrical equipment must conform to the standards in force and be earthed. Receptacles must be firmly closed and checked at intervals. They must be kept away from any heat source.

Persons from outside the laboratory may not enter this room which is classified as a **HAZARD AREA**. The key to this room is held by the supervisor who surrenders it to authorized persons only and ensures that the record of use sheet has been properly filled out. Special signs alert users or persons from outside the laboratory to the hazards involved.

2. SECONDARY STORAGE

Only persons authorized by the supervisor may enter the primary storage room; they must be given suitable protective equipment (overalls, gloves, mask, etc.).

When products are moved from the store cupboard to the working area for preparation of the solutions, they are transported in a container which is unbreakable and leak-proof if dropped. Inside it the bottles and flasks are packed in a bed of drying agent (e.g., vermiculite) or placed in racks above this absorbent material.

Where products have been stored in a refrigerator or ice chest the flask must be allowed to warm to room temperature before opening, to avoid a build-up of condensation inside the flask which could degrade the product and distort the sampling result in every case.

The operations of opening the container and flasks, transferring the primary flask to the target receptacle (which must have a screw-type closure) and preparing solutions of genotoxic, mutagenic and/or carcinogenic products, must be performed in accordance with the safety rules (cf. Chapter XII), over a sink which is resistant to the product and the solvent (enamelled or stainless steel, for example) and observing the normal precautions.

Following preparation of the solutions, the flasks are correctly labelled and tightly stoppered (no grinding in). **They must be transported only in hermetically sealed receptacles which will not break if dropped;** they must be packed in a bed of drying agent (e.g., vermiculite).

Receptacles containing flasks with genotoxic, mutagenic and/or carcinogenic products in solution are stored in the compartment reserved for them in a cupboard or a flameproof refrigerator or ice chest which is correctly marked and located in a ventilated room.

3. TRANSPORTATION OF CARCINOGENS

When a genotoxic substance has to be moved to another laboratory (within the same building), the risks inherent in transporting it must be minimized by packaging the product as follows:

- the product is placed in a tight screw-top tube, the top of which is held in place by sticky tape or parafilm;
- this tube is placed in a wide-mouth plastic jar filled with drying agent, itself closed by a screw top;
- the jar is placed in a shock-proof box[10].

When the product has to be transported to a point away from the immediate site, the rules in force since 1 January 1995 must be observed. These are those of the European Agreement (ADR) concerning the international carriage of dangerous goods by road of 30 September 1957 (and subsequent amendments), incorporated into French law by:

- the Order of 12 December 1994 on rules for the carriage of hazardous materials (rules on carriage by road and alphabetical classification of materials)[11]; and
- Decree No. 95-500 of 12 April 1995[12].

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[10] **Glass** trays must not be used! Receptacles of polycarbonate or stainless steel are acceptable. Polycarbonate has the advantage of being transparent, but if accidentally contaminated it must be disposed of with carcinogenic waste. Stainless steel can be decontaminated but is, of course, not transparent!

[11] Published in the *Journal Officiel de la République Française* on 27 December 1994.

[12] *Recueil des Documents Administratifs* No. 113 of 27 December 1994.

Annexes A and B to the European Agreement are reproduced in the two volumes of the *Recueil des Documents Administratifs* No. 113.

Annex A (volume I) sets out "*Rules concerning hazardous materials and objects*". By way of general rules, Annex A indicates which hazardous goods may be carried by road (and under what conditions) and which goods may not. Hazardous goods are divided into classes which are subject to limits (classes 1, 2 and 7) and classes not subjected to limits. The classes are as follows:

Class 1	Explosive materials and objects
Class 2	Gases which are compressed, liquefied or dissolved under pressure
Class 3	Flammable liquids
Class 4.1	Flammable solids
Class 4.2	Materials prone to spontaneous combustion
Class 4.3	Materials which give off flammable gases on contact with water
Class 5.1	Oxidizing substances
Class 5.2	Organic peroxides
Class 6.1	Toxic materials
Class 6.2	Infectious materials
Class 7	Radioactive materials
Class 8	Corrosive materials
Class 9	Miscellaneous hazardous materials and objects.

Genotoxic agents are covered by the rules for class 6.1 (unless they already belong to another class, e.g., in the case of radioactive molecules).

For each class of products the names of the products or the families concerned are quoted.

Certain toxic materials, carried under certain conditions (in particular in small volumes or weights), are not subject to the above rules. However, a number of minimum general packaging rules must be respected.

Annex B lists "*provisions relating to transport equipment and transport operations*".

This sets out in detail: the manner in which hazardous goods are transported (bulk, container, tanker, etc.). The special requirements which transport equipment must meet, general provisions (vehicle crews, special training for drivers, documents to be carried in the cab, etc.).

The special provisions applicable to each class are also listed.
Lastly, the rules of IATA are to be respected when products are forwarded by air.

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VI.

HYGIENE RULES FOR PERSONNEL INVOLVED IN THE HANDLING OF GENOTOXIC AND CARCINOGENIC PRODUCTS

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HYGIENE RULES FOR PERSONNEL INVOLVED IN THE HANDLING OF GENOTOXIC AND CARCINOGENIC PRODUCTS

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In areas where genotoxic and carcinogenic products are handled, **it is imperative to observe the following rules of hygiene:**

- personnel must wash their hands every time after handling a genotoxic product. Hands must be cleaned thoroughly, starting by rinsing in water, then washing in soap and water, and finishing by scrubbing the nails.
- Personnel must not work in street clothing without other protection.
- It is compulsory to wear overalls or special clothing, preferably impermeable, on top of street clothing.
- Overalls used in areas where genotoxic products are handled must be used in those areas only. There may be a system (colour, badge) to identify them readily if worn outside those areas.
- Gloves and goggles must be worn. Masks and overshoes are strongly recommended.
- Personnel may not drink, eat, smoke, apply cosmetics or store food or drink in areas where genotoxic products are handled.
- Fabric handkerchiefs may not be used, only disposable paper handkerchiefs.
- Nothing should be put in the mouth (e.g., pens).
- Pipetting by mouth is prohibited.
- Handbags or personal effects may not be brought into working zones.
- Animals, other than experimental animals, may not be present in working zones.
- The laboratory and working area must be tidy and clear of all items unrelated to the work in hand.
- Telephones, doorknobs, refrigerators, etc. must not be touched by a gloved hand which has handled carcinogenic or genotoxic products.
- Floors, walls and other surfaces must be cleaned regularly.

On the following page is a poster listing all these recommendations. It should be displayed in relevant work areas.

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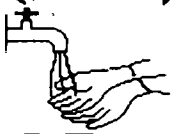
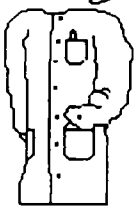
Book II of the Labour Code: Prevention of chemical hazard, section V, Chapter I, Title III, Decree No. 92-1261 of 3 December 1992.

HYGIENE RULES FOR PERSONNEL INVOLVED IN THE HANDLING OF GENOTOXIC AND CARCINOGENIC PRODUCTS

In areas where genotoxic and carcinogenic products are handled, **it is imperative to observe the following rules of hygiene :**



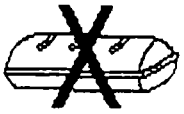
1. Personnel must wash their hands every time after handling a genotoxic product.
2. Hands must be cleaned thoroughly, starting by rinsing in water, then washing in soap and water, and finishing by scrubbing the nails.



3. Personnel must not work in street clothing without other protection.
4. Overalls used in areas where genotoxic products are handled must be used in those areas only.

5. Gloves and goggles must be worn.
6. Masks and overshoes are strongly recommended.

7. Personnel may not drink, eat, smoke, apply cosmetics or store food or drink in areas where genotoxic products are handled.



8 Fabric handkerchiefs may not be used, only disposable paper handkerchiefs.

9. Nothing should be put in the mouth (e.g., pens).
10. Pipetting by mouth is prohibited.

11. Handbags or personal effects may not be brought into working zones.

12. Animals, other than experimental animals, may not be present in working zones.

13. The laboratory and working area must be tidy and clear of all items unrelated to the work in hand.
14. Telephones, doorknobs, refrigerators, etc. must not be touched by a gloved hand which has handled carcinogenic or genotoxic products.

VII.

MEDICAL SURVEILLANCE AND BIOLOGICAL MONITORING

N. MAZALEYRAT, C. PLEVEN, F. PILLIERE

MEDICAL SURVEILLANCE AND BIOLOGICAL MONITORING

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Summary

European Directive 90/394 of June 1990, incorporated into French law in Decree No. 92-1261 of 3 December 1992, lays down a number of rules for prevention of the cancer risk.

The occupational physician and persons responsible for safety and health in the laboratory must together determine the procedures for surveillance and prevention to be put in place, taking account of the working conditions and the substances and concentrations used.

At present there seems to be no test to predict cancer; it is thus vital in every laboratory to insist that the safety rules designed to protect personnel are respected as fully as possible and that everyone is offered medical check-ups, so that the slightest change in health status can be detected and dealt with.

1. INTRODUCTION

Where genotoxic substances are used, a programme of medical surveillance must do several things: firstly, a maximum of prevention must be developed to protect personnel, and then effective methods of measuring exposure must be identified and used so that action can be taken at an early stage, which may still be deemed reversible, even before the appearance of any body lesions.

Examinations must be simple, non-aggressive and thus readily accepted and repeated, their frequency being weighted according to the risk factors involved; they should also yield precise and reproducible results.

The Labour Ministry circular(1) acknowledges the difficulty of this kind of monitoring since: "carcinogens are difficult by virtue of their insidious, abstract nature and because the processes whereby they are identified are slow to develop". Medical surveillance thus faces a number of difficulties:

a) "Background noise" from spontaneous cancers;
b) The multifactoral aspect of cancers caused by the combined action of a number of carcinogens, synergistic effects (e.g. smoking, alcohol, etc.);
c) The different effects of several toxic substances handled;

d) The time lapse between exposure and effects.

Effective medical action must take account of the mechanisms of toxic action, the frequency and severity of exposure and the sensitivity of the individual.

According to Dolls (2), quantitative estimates of the degree of risk entailed by a specific exposure have been made only for ionizing radiation.

At present, the relationship between chemical substances and target organs is known in a few cases. This knowledge serves as a guide for medical surveillance and examples are given in Tables 2 and 3.

Thus the work of the occupational physician must include participation in epidemiological studies in order to identify the effects of new products.

Table 2

SUBSTANCES	**BIOLOGICAL PATHOLOGY**	**SURVEILLANCE**
Aromatic amines	Bladder cancer	Cytological investigation
Aflatoxin	Liver cancer	Liver values: BSP
Vinyl chloride	Liver cancer	Alkaline phosphatase Transaminase
Benzene	Leukaemia	Cell count Blood formula

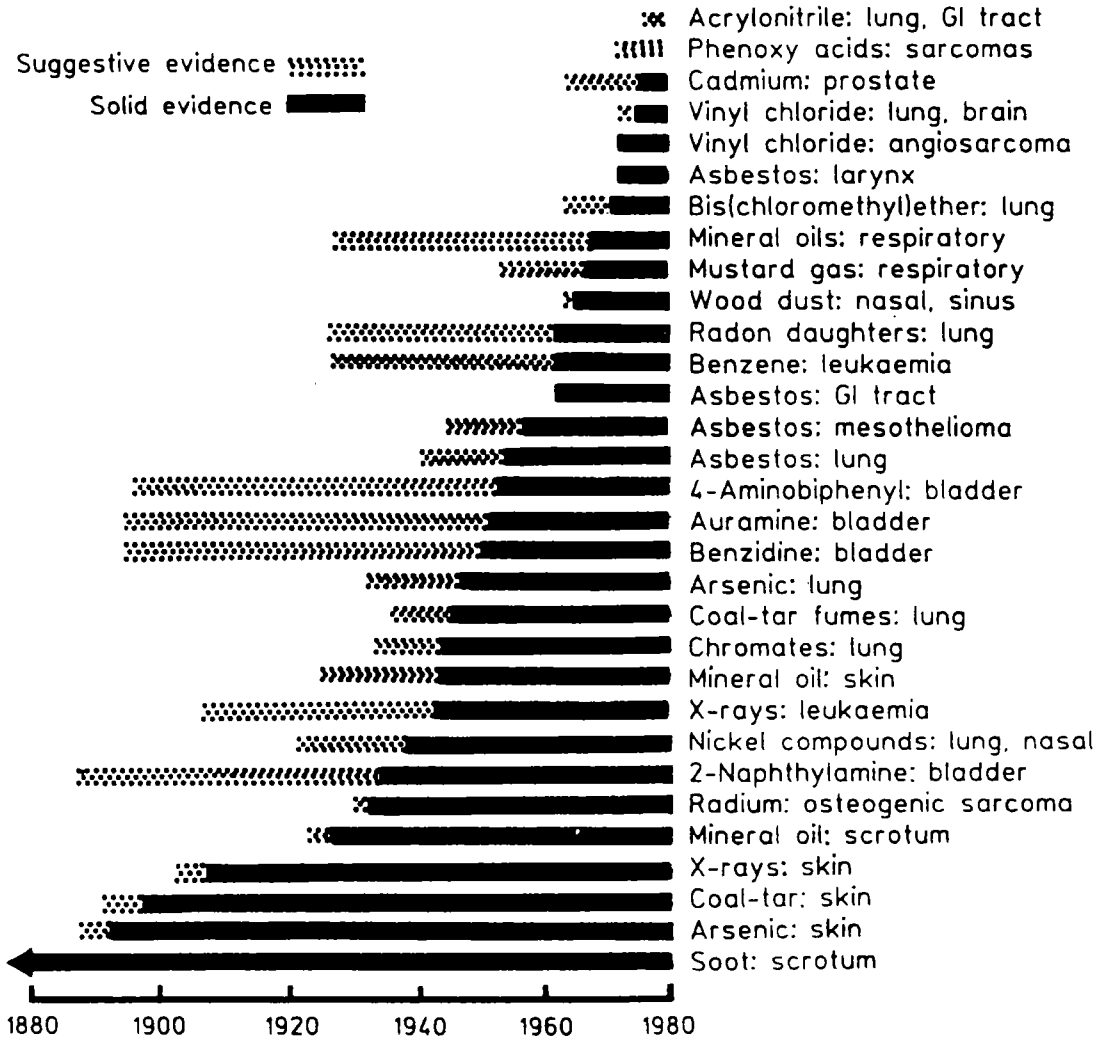
2. PRIMARY PREVENTION

The object of this is to detect the presence of carcinogenic substances at the workplace (in the ambient air or on work surfaces). This may be demonstrated by passing an adequate solvent over a work surface and then measuring (e.g. HPLC). Analytical procedures should use sensitive methods and be validated in competent laboratories (4).

The results enable immediate changes to be made to working conditions in order to minimize the exposure of personnel.

Wherever possible, the replacement of genotoxic products by ones which are less toxic should be suggested.

Table 3. Occupational cancer – historical development of evidence
(from Vainio, 1987)



3. SECONDARY PREVENTION

3.1 Introduction

This identifies any penetration of substances into the body, by demonstrating genotoxicant body burdens in a group of individuals.

Certain changes in biological constants, whilst not necessarily presaging a tumour, enable us to quantify exposure to substances and even to identify early biological effects.

The methods used must meet a number of criteria and must include several of the following features (5):

- they must be suitable for monitoring exposure and its effects on health;
- they must be validated by individual or group results;
- they must be reproducible, specific and without risk to the subject;
- they must have detection limits and show inter- and intrapersonal variations, variations within a non-exposed population caused by endogenous factors (age, sex, race) or exogenous interference factors (diet, smoking habit, alcohol use, physical stress);
- tests must be performed in laboratories with experience in genotoxicity testing.

NB:

- Variation in individual responses may occur simply because the population is heterogeneous;

- Negative findings may mean that the exposure level is below the sensitivity range of the test, not that the product is harmless;

- Positive findings for a group must be followed by changes to their working conditions.

3.2 Possible methods for detecting exposure to mutagens (7, 8)

3.2.1 Changes in blood and/or biological parameters

Principle
- Haematology screen (blood volume, leukocyte count and formula);
- Liver values (prothrombin, alkaline phosphatase, transaminase);

Example: method used to assess the effects of exposure to certain solvents.

Advantage
- Easy to perform.

Limitation
These values are altered in many types of pathology. However, a gradual rise in alkaline phosphatase over successive tests, for example, is indicative of disease.

In all cases, the results must be regarded only as qualitative markers of exposure to a genotoxic agent.

3.2.1 Measurement of genotoxicants and/or their metabolites in biological media

Principle
Genotoxicants or their metabolites can be measured in biological fluids.

Correlations have been established for certain substances between atmospheric concentrations and biological concentrations (in blood or urine).

3.2.1.1 Urinary and/or blood measurement

The BIOTOX document (9) gives a non-exhaustive list of laboratories which conduct biological measurement (blood or urine) of industrial toxicants. These include a number of genotoxicants, with information on their metabolism.

Example: benzene, benzidine, formaldehyde, trichloroethylene.

Advantage:
Biological fluids (blood, urine) are easily collected.

Limitations
- method specific to one agent
- usually checks only recent exposure, except in the case of cumulative toxicants.

Laboratories conducting these measurements
See BIOTOX document (9).

3.2.1.2 Urinary mutagenicity (Ames assay, SOS chromotest)

Principle
Detection of mutagenicity in biological fluids.

- ***Ames assay in urine***

The genotoxic agent or its urinary metabolites induce mutations in strains of *Salmonella typhimurium*, usually strains TA100 and TA98 only.
The urine has to be concentrated first.

- ***SOS chromotest***

Using urine, induction of the damage repair system is demonstrated by a colorimetric test on *E. coli* PQ 37.

Advantage

Urine is easy to collect, the test is quick and it can be used for screening purposes.

Limitations

- Urinary mutagenicity persists for 1 to 2 days;
- The genotoxicant has to be eliminated in an active or activable form;
- Not very sensitive and not validated in the case of the SOS chromotest;
- Possibility of interference factors such as smoking;
- Methods do not allow genotoxicant reactivity to biological macromolecules to be assessed (RNA, DNA proteins).

3.2.3 Genotoxic activity in the body

Genotoxic activity may be apparent:

- either in the macromolecules of the body, by adduct formation or stimulation of enzyme repair systems;
- or in the cells by the presence of cytogenetic anomalies.

These measurements are useful in that they reflect the immediate consequences of exposure. At present, however, it is difficult to assess the risk to an exposed individual's health on the basis of positive findings for these tests.

3.2.3.1 Detection of adducts on cell DNA, erythrocytic haemoglobin or serum proteins

Principle

Genotoxic agents bond themselves covalently to the nucleophilic sites of macromolecules and are identified by post-labelling or radioimmunology techniques (e.g., benzo[a]pyrene, acetylaminofluorene) or by immunoenzyme measurement.

a) Protein adducts

Bonding to the nucleophilic sites of amino acids:

Adducts can be studied:

- in serum proteins: this technique tests the blood monocytes and can asses short-term exposures;
- in haemoglobin: this method tests the erythrocytes and identifies former exposure and/or cumulative exposure (the half-life of erythrocytes being 3 months).

For examples of adduct determination in humans or animals as a method of monitoring exposure, see bibliography references 10 (ethylene oxide) and 11 (aromatic amines).

i) Advantages
- Sensitive method;
- Adducts are more stable than DNA adducts, their disappearance depends on cell renewal and DNA repair capability;
- Target cells are easily obtained;
- For proteins synthesized in the liver cells there is a good capacity for metabolism of indirect alkylating agents.

ii) Limitations
- No direct mutagenic effect can be measured, but for certain products (e.g., ethylene oxide) a correlation with DNA adducts has been established;
- Technique is complex and not validated;
- Procedure is unwieldy and difficult to apply routinely;
- Does not detect molecules with an affinity for cells other than blood cells (e.g., safrol).

b) DNA adducts

Bond to the nucleophilic sites of nucleic acids.
Adducts usually measured on the DNA of monocytes.
For an example of adduct determination in the case of polycyclic aromatic hydrocarbons, see bibliography reference 12.

i) Advantages
- Sensitive method, the detection limit can be 1 adduct per 10^9 to 10^{10} nucleotides;
- In some cases a dose-effect relationship makes dosimetry possible;
- Target cells are easily obtained;
- Measurement of impact on DNA, the target macromolecule for mutagens;
- Specificity of the genotoxic agent.

ii) Limitations
- Technique is unwieldy, expensive, not validated at the development stage and is specific for the formation of certain adducts (the use of radioimmunological techniques means that specific antibodies have to be used for each adduct);
- Interpersonal variation which may depend on the balance between the mechanisms of metabolic activation and deactivation (genetic polymorphism of acetyltransferase activity) or on DNA repair systems;
- Possibility of interference factors (e.g., smoking);
- Does not detect molecules which have an affinity for cells other than blood cells (e.g., safrol).

3.2.3.2 Cytogenetic tests on lymphocytes, erythrocytes or target cells

Three types of tests are used to identify clastogenic activity:
- detection of chromosomal aberrations at metaphase;
- detection of micronuclei;
- detection of sister chromatid exchanges.

The choice of test to verify exposure to genotoxicants will depend, among other things, on the degree of the subject's exposure and the latency period between exposure and analysis.

In all cases, the results must be interpreted with caution because:

i) "It is generally accepted that the general significance of a positive cytogenetic test is not understood";
Indeed, one cannot assume that a higher cytogenetic test result means a deleterious effect on health.

ii) "The only conclusion one can draw from an increase in chromosomal aberrations is that the cancer risk for the group in question may be higher". If one is seeking to define a risk, this must be done for a group and not for individuals. To improve assessment, an individual's responses to cytogenetic tests should ideally be known before he is exposed.

a) Detection of chromosomal aberrations by analysis at metaphase

Principle

Structural chromosomal aberrations are determined in peripheral blood lymphocytes by microscopic observation at metaphase following *in vitro* induction of cell division.

Advantages

- Old and proven technique;
- Clastogenic effect persists for a number of years and can thus be used to demonstrate cumulative effects.

Limitations

- Not a very sensitive method;
- Methodological difficulties or differences in susceptibility to chromosome damage and ability to repair it make it impossible to verify any dose-effect relationship;
- Inter-laboratory reproducibility is poor;
- Possibility of interference factors (e.g., smoking, use of pharmaceuticals, pathological conditions, etc.);
- Unwieldy to carry out.

b) Detection of micronuclei

Principle

Identification of a whole chromosome or fragment in the cytoplasm of cells such as the lymphocytes, erythrocytes or epithelial cells. This is due to chromosome breaks or segregation defects during mitosis.

Studies have been conducted on workers exposed to ethylene oxide or styrene.

Advantages

- Simple and quick technique;
- Possible to work on target cells;
- Prompt analysis after exposure detects acute exposures.

Limitations
- Past performance cannot be judged since use of the technique is relatively new;
- Micronuclei can be induced by a range of pathologies (e.g., sickle cell anaemia).

c) Detection of sister chromatid exchanges
Principle
Observation in lymphocytes, after growth in the presence of bromodeoxyuridine and staining of the two chromatids, of exchanges of material between the two DNA helices. These exchanges are the cytological reflection of damage to the DNA.

Advantage
Sensitive indicators of genetic damage.

Drawbacks
- Of limited value for verifying long-term exposures, as sister chromatid exchanges persist for a few weeks only;
- Technical factors may cause variability in responses;
- Possibility of interference factors (smoking, use of pharmaceuticals, genetic susceptibility);
- Possible variations in interpretation depending on whether cells with a high frequency of sister chromatid exchange are taken into account.

d) Comet assay
Principle (15)
Microelectrophoresis technique allowing visualization and quantification of primary DNA lesions (single and double strand breaks and weak alkaline sites) in individual cells.

Advantage
Simple technique, yields prompt results.
Responses for 12 products reported in references
(has been tested for X-rays, heavy metals, anticancer drugs, ref. 16).

Drawbacks
Test little used at present.
Currently being standardized and validated.
High initial investment (e.g., imaging equipment).

3.2 Early detection of cancers

Since prevention cannot be total, medical surveillance has a role to play in detecting precancerous lesions and tumours as early as possible.

Detection methods must be sensitive and specific.

3.3.1 Classic examinations (17)

*a) **Radiological examinations***
- computerized axial tomography, X-ray;
- scintigraphy;
- echography, scanner, NMR.

Limitations
Cannot detect small tumours, except NMR (MRI). The latter method can be used for brain, lung and liver tumours of 2-4 mm diameter (18, 19).

*b) **Cytological examinations***
Identifies lesions in the lung (cytological analysis of sputum) or bladder (urinary smear).
Examples: Urinary smear, reliable method used for workers exposed to aromatic amines.

Limitations
These methods cannot detect risks for low levels of exposure (9).
- Methods are time-consuming and expensive;
- Few services equipped to carry them out.

*c) **Haematology screen***
Identifies neoplasms in the haematopoietic tissues (leukaemia, lymphoma, etc.)

3.3.2 Biological markers of cancer

*a) **alpha-foetoprotein***
This marker cannot play a part in early detection.

*b) **carcinoembryonic antigen (CEA)***
Measurement of CEA is not very useful for early detection since it is neither sensitive nor specific for cancerous lesions.

*c) **γ-glutamyltransferase***
This enzyme is not specific for neoplasms.

*d) **monoclonal antibodies***
A range of problems arise when these techniques are used, one problem being that the specificity of antibodies used to date is only relative.

Biological markers were thought promising at first but would seem to be of little value for the early detection of individual cancers because of the difficulties of interpretation and because they are as yet insufficiently understood.

4. ASSIGNMENT OF PERSONNEL

Before a definite decision is taken to assign an individual to work in the laboratory, the laboratory supervisor must, to the best of his ability, assess the applicant's personal qualities:
- technical competence;
- professionalism;
- mental stability;
- team spirit;
- personal hygiene.

The occupational physician must issue a statement of aptitude based on the individual's occupational and medical history and must make a full medical assessment (with blood tests and, if possible, a genotoxicity study to serve as reference).

The occupational physician may liaise with a specialist team to determine the suitability of an individual who has a chronic disorder, long-term drug treatment (corticoids, antibiotics), immunodeficiency, physiological or genetic predisposition.

Pregnant women must declare their pregnancy at the earliest possible stage to the occupational physician who will take all necessary measures to eliminate risk.

5. RECORDING OF INFORMATION AND KEEPING OF A MEDICAL RECORD BOOK

In each laboratory, the following information should be collected and made available to the occupational physician and the health and safety committee/works council:
- names of personnel, with the dates they began and stopped working in the department;
- job descriptions;
- names of carcinogens used;
- approximate frequency of exposure;
- pathways into the body (inhalation, skin, oral), extent of exposure, in the event of an accident;
- dates and duration of absences and reasons.

This information is essential in order to tailor detection methods as appropriately as possible to the substances involved, in conjunction with the medical record book which states:

- dates and results of exposure measurements;
- dates and results of specific check-ups and tests.

Since the effects of contamination may only become apparent long after the time of exposure, this information must be kept for 40 years after exposure ceases (Article R 231-56-11) and medical surveillance should continue even after an individual changes job, spends a period of time off work or ceases work altogether.

In future years this information should ideally be centralized, so that databanks can be set up and epidemiological studies can be conducted later with a view to better evaluation of the occupational hazard faced by persons working in research laboratories.

6. CONCLUSION

The immediate and prime objective of medical surveillance is to monitor the individual and collective protection given to personnel and to improve working conditions. It will also help in the conduct of epidemiological research, since this alone can identify and quantify the risk inherent in genotoxic substances and find ways of controlling it.

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VIII.

HANDLING OF CARCINOGENIC OR MUTAGENIC PRODUCTS

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HANDLING OF CARCINOGENIC OR MUTAGENIC PRODUCTS

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As there are few ways of directly monitoring contamination, exposure to a carcinogenic or mutagenic substance must be ***systematically avoided*** *in order to minimize the hazard both to oneself and to others.*

A number of protective measures must thus be rigorously observed when hazardous substances are handled.

No operation in which a mutagenic or carcinogenic product is handled may be carried out unless a detailed written protocol has first been drawn up which includes considerations of individual and collective protection.

1. PRODUCT WEIGHING

1.1 Premises

This section recapitulates some of the details set out in Chapters III, IV and V. The critical weighing stage can be avoided, in some cases, by purchasing ready-weighed products in sealed packagings, or it can be limited by the use of prepared aliquots of fractionated solutions stored at -20°C.

If weighing is unavoidable, a room must be set aside specially for the purpose and for subsequent handling operations such as solution preparation and storage. This room is a controlled access area and must :

- have an airlock in which the operator can put on his protective equipment;
- be properly ventilated (access to it is prohibited during handling, perhaps with a red light positioned outside the room);
- be specially equipped for:
  . sampling and transfers (fume cupboard, ventilated chemical hood);
  . storage (refrigerator, ice chest, cupboard);
  . weighing (balance);
  . waste (storage containers marked "carcinogenic waste").

This room must be marked with the "chemical carcinogens" logo and must be kept locked.

1.2 Personal protection

This section recapitulates some of the details set out in Chapter IV. The following conditions must be observed:

. double pair of single-use gloves, to be chosen for its suitability for the type of product handled;
. overalls or single-use jacket;
. single-use overshoes;
. organic dust or vapour mask;
. protective goggles.

The outer pair of gloves must always be put on and removed under the safety hood.

1.3 Weighing

1. Before a flask stored in the refrigerator or ice chest is used it should be allowed to return to room temperature in order to avoid condensation when it is opened (which may distort the weighing result).

2. In every case, substances must be weighed by difference, i.e. by double weighing in a (previously tared) glass receptacle with a hermetically sealed stopper. Do not try to adjust the weight exactly by adding or removing substance. The balance must be placed away from personnel traffic areas, in a draught-free location. Use of ventilation during weighing can disperse fine powders in the surrounding air and may also distort the weighing result. In such cases the ventilation must be switched off.

    1) tare the stoppered weighing receptacle;
    2) introduce an approximate quantity of product under the hood;
    3) close the weighing receptacle under the hood;
    4) weigh.

3. Substance may be transferred into the weighing receptacle:
    . under a ventilated chemical hood;
    . under a fume cupboard with collection at the exhaust point;
    . in a glove box with air recirculation, fitted with HEPA and activated carbon filters.

Transfer of volatile liquids must be done under an adequately ventilated hood (0.5 m/sec).

4. Solids should ideally be transferred using single-use spatulas. (**Note:** some of these spatulas are sterilized in ethylene oxide which can interfere with the work).

Liquids should be transferred using a syringe or positive displacement micro-pipette.

The outer pair of gloves should be replaced promptly if it becomes soiled or at regular 30-minute intervals if the weighing operation is a lengthy one.

1.4 Recording of weighing results

Every weighing operation must be entered in a register kept especially for the purpose, stating the product name, the date weighed, the quantity weighed and the name of the person doing the weighing.

2. PREPARATION OF SOLUTIONS IN SEALED FLASKS

When solvent is injected into sealed flasks it is essential to make a pinhole to let the air out and equalize the pressure so that nothing will splash out when they are opened. This operation must be performed under a safety hood.

3. WORK SURFACE

The work surface must be clear of all non-essential objects or products so that mishaps can be avoided and the area can be decontaminated in the event of an accident. It must be covered by an absorbent paper, the underside of which is sealed by a plastic film (Benchcoat). It may be preferable to work over a sink, to limit the area which needs to be decontaminated.

All the necessary transfer equipment (spatula, single-use pipettes, tubes or subsample receptacles, absorbent paper or drying agent) must be prepared and placed on the work surface beforehand.

All soiled equipment must be placed directly in a plastic sack provided for the purpose and positioned inside the workplace.

After handling;
. the absorbent paper from the work surface must be carefully folded and placed in the plastic sack:
. the sink or work surface must be carefully cleaned with alcohol or an appropriate solvent using absorbent paper which is then disposed of in the plastic sack
. the plastic sack must be disposed of in the carcinogenic waste container;
. the safety hood must be left switched on for at least 30 minutes.

4. OPERATOR MOVEMENTS

These must be precise and controlled to ensure that no extraneous contamination of receptacles can occur. In the case of a new experiment or a novice operator, it may be useful to start with a blank experiment, perhaps using a bright stain or fluorescent product which will show up even the tiniest areas of contamination. Contaminated

surfaces must on no account come into contact with clean surfaces. If in doubt, clean the surface with paper very carefully several times, using alcohol or an appropriate solvent and replacing the paper several times.

5. PIPETTING

Since solutions must not be transferred by mouth, manual "propipettes" set aside especially for this purpose must be used, or alternatively an electric pipetting system of the "Pipet-aid" type for large volumes and micro-pipettes with disposable tips for small volumes. All these pipetting units must be marked, must be used for this purpose only and must not leave the work station.

When using micro-pipettes, care should be taken to avoid the formation of aerosols due to bursting of the bubbles which can form in solutions of high surface tension.

6. PREPARATION OF SOLUTIONS OR SUSPENSIONS

Preparation of solutions or suspensions must be done under a protective hood. Successive dilutions must be prepared in hermetically sealed glass or plastic receptacles, previously marked with the product's name and its concentration or quantity and the solvent used. Lastly, the tubes must be placed in compartmentalized boxes and marked for storage at 4°C or -20°C.

7. MOVEMENT OF PRODUCTS OR PRODUCT SOLUTIONS OR SUSPENSIONS WITHIN THE LABORATORY

Where possible products should always be moved as solutions, never in powder form. They should be transported in boxes (metal or plastic) which are hermetically sealed and contain a drying agent in case of breakage (vermiculite or universal drying agent).

8. SPECIAL PRECAUTIONS DURING ASPIRATION OF CULTURE MEDIA

To begin with, volumes of culture medium to which a carcinogenic or mutagenic product has been added should be drawn off manually using a small-volume "Pipet-aid" and should be stored in a disposable receptacle to be discarded and processed in the same way as contaminated solutions. The flask used to hold the solutions needs to be incinerated as it stands. Addition of an antiseptic (e.g., sodium azide) prevents any proliferation and any risk of leakage from the flask as a result of a rise in pressure due to gas formation.

Later on, subsequent washing of the preparations must be done using suction apparatus especially reserved for the purpose. Care must be taken to fit the suction hose with a retaining bottle. The aspiration receptacle and hose must be carefully decontaminated after use. The washing liquids must be treated as contaminated liquids.

Conditions of storage and disposal must take account of other hazards (radioactivity, biological hazards, etc,).

IX.

SAFETY IN ANIMAL EXPERIMENTS

B. MOLINIER & M. CASTEGNARO

SAFETY IN ANIMAL EXPERIMENTS

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1. INTRODUCTION

Contamination of animal quarters is unavoidable and every effort must be made to minimize it. Thus, Sansone and co-workers showed that:

1. The preparation of feed *containing genotoxicants* led to contamination not only of the equipment used to prepare it but also of the equipment for transporting, protecting and administering it, together with contamination of the ambient air and the floors and walls of rooms in which feed is prepared (Sansone et al., 1977).
2. Addition of a volatile product to animal feed leads to contamination of the ambient air and of the entire room in which this feed is given, especially the cages (Sansone & Losikoff, 1978).
3. Administration of contaminated feed causes more contamination if it is given in cages with a wire bottom than in solid-bottom cages (Sansone & Fox, 1977).
4. Unless drastic measures are taken, contamination from animal quarters can spread to adjacent premises (Sansone & Losikoff, 1979).

It is apparent from the above that the safety procedures required for *in vivo* studies will be shaped essentially by what is known about the physicochemical properties of the product being studied and its toxicity, and our knowledge of these increases as research on them evolves. These safety procedures also depend on the stage that the operator has reached in his experiment. We shall thus examine these three points.

2. PRECAUTIONS WITH REGARD TO THE CHEMICAL/PHYSICOCHEMICAL PROPERTIES OF PRODUCTS

Firstly, and very briefly, the important chemical and physicochemical information which an operator needs to know before working on a product is as follows:

- *its reactivity with a given vehicle: e.g., certain thiocarbamates dissolved in water give off phosgene; ammonia may be used to neutralize this;*
- its stability alone and in solution or suspension;
- its vapour pressure at storage temperature and room temperature, to avoid any risk of inhalation;

- its physical state: products are more easily dispersed if they are powders or small electrostatic particles.

In any event, and in the interests of prevention, it is preferable in every case to store products in a properly ventilated place (cupboard or cold room), to wear a cartridge mask in order to avoid breathing in any product vapours when these cupboards are opened, and to wear goggles and gloves to avoid contact with eyes and skin.

The product may be a solid, liquid or gas. Volatile products should preferably be administered by injecting solutions or by gavage; but this sometimes leads to the formation of aerosols and mixing them in with the feed causes evaporation or the formation of aerosols. In every case, these operations must be carried out under well-ventilated hoods and the animals must be held in ventilated enclosures. With solid or liquid products, the methods of preparing the product, i.e. as a solution or suspension, and the method of treating the animals with it will be more or less the same. With gases and aerosols the preparation process is different. One essential precaution is to avoid leaks in the inhalation system and blow the system through completely with air when the exposure chambers are opened so that vapours cannot be inhaled. During handling the air is cleaned as it leaves the cage by filters capable of adsorbing the products concerned. Lastly, if the product can be exhaled, the animals are placed, outside the treatment periods, under a hood which has a face velocity of at least 0.5 m/sec.

Following this rapid review of physicochemical information, we shall now consider the toxicological information available, which depends on the stage of development of the product.

3. PRECAUTIONS WITH REGARD TO THE PRODUCT'S TOXICITY

When a product is first developed its potential toxicity is not known. The first studies of it are usually acute toxicity studies (toxicity testing after a single administration), studies of local tolerance (irritation to skin and eyes), and studies of sensitization and mutagenicity. In these cases, maximum precautions must be taken during handling.

In the case of a non-toxic, non-mutagenic product the precautions may be less stringent. Nevertheless, it is worth remembering that certain products which demonstrate no mutagenicity have been shown to be carcinogenic in animals and/or man.

Maximum precautions must be taken in the case of a product which irritates the skin and/or eyes or sensitizes.

Pregnant women must not be involved in any way with the treatment of animals.

Maximum precautions must also be taken in the case of mutagenic products or a product which is a known carcinogen, e.g., the positive controls used in *in vivo* mutagenicity studies, or a suspected carcinogen – something which will only be known once carcinogenicity studies on it are completed.

4. CONCEPT OF MAXIMUM OR MINIMUM PROTECTION DURING PRODUCT PREPARATION

The concepts of minimum or maximum protection and the recommended precautionary measures will depend on the stage which the operator has reached in his experiment. Briefly, these are three in number: the site where the product is prepared, its transportation to the animal areas and the animal experiments *per se*.

Firstly, the product will be stored at the site where solutions are prepared. As we saw in Chapter III, this is an isolated and properly ventilated room. There are various types of equipment, depending on the type of preparations required and the toxicological information available: in the case of products whose toxicity is not known, solutions or suspensions of them are prepared under a hood fitted with a mobile suction hood which draws off the air at the bottom, filters it and vents it to atmosphere; the preparer wears latex gloves (liquid products or solutions) or cotton gloves (small electrostatic particles), sleeve protectors, a protective mask and goggles.

For products which are non-toxic, non-mutagenic and non-carcinogenic, minimum precautions are observed during weighing and preparation: gloves, protective mask and goggles.

For mutagenic products, the maximum precautions described for products of unknown toxicity are observed. When they are not handled on the vacuum pick-up table, the operator wears a disposable protective apron and sleeve protectors on top of his overalls (**Note: plastic should be avoided when handling powdery substances**).

Maximum precautions must be observed when handling teratogenic products or products which affect reproduction or certain hormone derivatives, and this handling must not be done by pregnant women or nursing mothers.

In the case of toxic and highly hygroscopic products, e.g., a vasodilator causing a flush, the operations of weighing and solution preparation may be done in a glove box. (**Note: care should be taken with transfer via gloves and the glove box which is a permanently contaminated enclosure**.) Every precaution must be taken (decontamination, changing gloves) before the glove box is used again.

The preparation of contaminated animal feed is a maximum hazard stage, as Sansone & Fox (1977) showed. Maximum precautions must be taken during preparation: inside an airlock, the operator puts on overalls, a cap, gloves, goggles, work shoes or single-use overshoes before entering the "mixing room". The powdered feed is placed in a distributor and sent by a pneumatic system to the various mixers, the quantity of feed and its destination being controlled by the operator from a microcomputer. During this time the product is weighed and pre-mixed by a pestle in a food mortar; the pre-mix is then introduced into the chosen mixer where the whole is shaken and homogenized; the preparation is then placed in bags which are heat-sealed and taken to the animal quarters. Contact between operator and product is thus truly minimal.

Gelatine capsules of powdered feed are now used less and less. They are not a good idea, partly because of the risks of products breaking down during manufacture and, secondly, because of the problems in sterilizing the capsules: autoclaving is not acceptable and neither is dry heat, which may lead to evaporation or decomposition of genotoxicants; irradiation in external structures carries a risk of contamination and possible degradation.

5. TRANSPORTATION OF PRODUCTS TO THE SITE OF ADMINISTRATION

When preparations are ready, product solutions must be transported to the animal quarters in sealed receptacles which are proof against products and solvents alike; preparations may be placed in tubes with screw-top closures and aluminium foil liners, which are then placed in closed plastic boxes or hermetically sealed stainless steel carrying containers of the kind recommended by IARC. Sacks containing feed mixes are moved in trolleys.

6. ANIMAL TREATMENT

The precautions observed have three objectives. The first is to protect the experiment, i.e. the animal's health (there must be no microbial contamination) and its environment, because the work is being done in a special (marked) room. The rate of air change in these rooms is high, about 12 per hour, i.e. a full air change every five minutes. Obviously, this means that fine particles of powdered feed are likely to be swirled up into the air and also increases the risk that these particles may be inhaled where the treatment route is feed, or that aerosols may be inhaled where the treatment is by gavage.

The second objective is to protect the researcher against biological hazard, which we shall not explore further here, and chemical hazard.

The third objective is to protect the environment against product dispersion.

In order to meet these imperatives at all times, when an operator enters animal quarters he passes through a first airlock where he undresses, a second airlock where he washes his hands and forearms and a third airlock where he puts on cotton trousers and jacket, overshoes, a cap and a paper mask (a ventilated helmet is recommended rather than the cap and gloves for a protracted exposure). Inside each examination room, moreover, he puts on fabric overalls, paper overshoes, gloves and possibly sleeve protectors to cover the wrist between sleeve cuff and glove. Fabric clothing must be changed at least weekly, and paper clothing must be discarded after every use. When leaving, in order not to spread contamination outside the animal quarters, the researcher must leave his clothes in airlock No. 3, moving on to airlock No. 2 where in every case he takes a full shower.

In the case of studies using a feed mix, as we have seen previously, the product is incorporated in a very fine powder. In order to minimize the risks of inhaling this powder, feeding trays must be filled under a hood. A ventilated helmet must also be worn when feeding trays are being changed and hooked up in cages. The clothing worn is thus the classic clothing for hazard areas: goggles, gloves, overshoes and overalls. Furthermore, in this kind of study solid bottom cages rather than wire bottom cages must be used to reduce contamination of the environment.

7. DECONTAMINATION OF EQUIPMENT

After treatment, the beakers containing left-over solution or suspension are returned to the preparation site where solutions are disposed of, together with the first washing liquid, in drums which are provided for the purpose and then stored in larger containers pending removal by an outside waste processing company. They may also be disposed of as described in Chapter X. A cage cleaning system must be located close to the animal quarters to reduce the contamination risk caused by transporting contaminated cages. Cages contaminated with carcinogenic substances must in every case be decontaminated before being washed.

Contaminated bedding and cadavers must be disposed of as described in Chapter X.

Animal quarters must be cleaned by the animal handlers themselves, who will have been suitably trained in treating contaminated surfaces and will be provided with all necessary protective equipment. Cleaning must be done in such a way as to avoid the generation of dusts or aerosols. **Consequently no cleaning using dry methods is permitted.**

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X.

METHODS FOR COLLECTION AND DISPOSAL OF MUTAGENIC AND CARCINOGENIC WASTE FROM RESEARCH LABORATORIES

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METHODS FOR COLLECTION AND DISPOSAL OF MUTAGENIC AND CARCINOGENIC WASTE FROM RESEARCH LABORATORIES

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Research into cancer and mutagenesis necessarily means handling carcinogenic/mutagenic substances and consequently the possible contamination of equipment, floors and work surfaces together with the production of potentially carcinogenic/mutagenic wastes which must not be released into the environment without prior treatment. Before such products are used, it is thus necessary to have a protocol for the collection and processing of wastes contaminated by these substances or their metabolites. Metabolites in fact are sometimes more carcinogenic than the products from which they derive. In addition to the protocol on waste there must be a protocol on emergency procedures for decontaminating floors or other soiled surfaces (see Chapter XI). A similar problem arises with the handling, in hospitals or by domiciliary personnel, of a large number of drugs, especially those used in cancer chemotherapy. It is imperative that every supervisor of a research laboratory or diagnostic and therapy service or every industrialist who manufactures, conditions or uses products which are potentially carcinogenic/mutagenic should inform their personnel fully of the safety rules to be observed when these products are used and should put in place arrangements for disposing of the waste from these handling operations.

1. LEGISLATION ON WASTE

The first requirement for proper waste disposal is familiarity with the relevant laws and regulations. The principles for managing genotoxic wastes are the same as those for other wastes and must be consistent with the general legislative requirements. They may be summed up as follows: **choice of products – sorting – conditioning – collection – storage – transportation – processing.** It should be pointed out, however, that **in the context of occupational hazard prevention** requirements in respect of the collection, storage and safe disposal of waste are only explicitly stipulated for products which are carcinogenic.

1.1 General legislation

Law No. 75-633 of 15 July 1975, as amended in 92-646 of 13 July 1992, concerning waste.

This lays down the definition of waste, the **responsibility** of the producer and the principle that the **"polluter pays".**

The Law makes producers of waste responsible for it up to and until it is eliminated altogether, and defines waste as follows: "any material or residue of production, transformation or use, any moveable item which the holder has discarded or intends to discard."

The Law defines the concepts of **"final waste",** within the current limits of our understanding, and of **proximity.**

It also requires the volume and harmfulness of wastes to be reduced, **reprocessing procedures** (recycling, re-use, etc.) to be put in place and plans for waste management to be drawn up.

Law No. 76-663 of 19 July 1996, amended: on classified facilities for protection of the environment. Decree 77-1133 of 21 September 1977, amended by Decree 94-94-484 of 9 June 1994.

This Law

e) applies in particular to producers of waste who have their own waste processing facilities *in situ;*
f) requires establishments with such processing facilities to comply with an administrative procedure of reporting or licensing;
g) empowers the Prefect to rule on classified facilities within his area of authority.

Circular of 14 June 1994: merging of the nomenclatures of classified facilities for protection of the environment.

Order of 25 January 1991 on facilities for the incineration of urban residues:

This text is concerned with the prevention and reduction of atmospheric pollution and sets a timetable for bringing incinerators into line with the law.

These imperatives make it difficult for producers to have approved waste incineration facilities of their own and encourage them to use companies which specialize in waste processing.

Law 92-3 of 3 January 1992 on water.

Water is regarded as a natural asset and the law requires the 6 water companies to provide technical, financial and economic assistance to anyone producing waste which may disturb the environment of natural water resources, in order to avoid pollution, in particular by providing appropriate information.

Law 95-101 of 2 February 1995 on protection of the environment.

Directive 94-67 of 16 December 1994 (European Union) concerning the incineration of hazardous waste but excluding incinerators for animal carcases or remains and infectious clinical waste.

1.2 Specific legislation

Annex 1 to the ***Order of 4 January 1985*** on the monitoring of systems for the disposal of pollutant wastes lists the categories of waste concerned and, where the quantity of this waste produced is greater than 100 kg per month or when a load is larger than 100 kg, requires the producer to issue a **waste consignment sheet** when the waste is surrendered to another party. This sheet gives precise details of the provenance, characteristics and destination of the waste, and travels with it to its ultimate destination which may be a final disposal facility, a collection facility or an interim processing centre.

Directive 67-548, amended by Directive 91-689 of 12 December 1991 (European Union) on "hazardous waste".
This defines the characteristics of "hazardous waste". It has one or more characteristics requiring labelling on the basis of Directive 67-548. Hazardous waste is thus waste with the following characteristics:

Explosive	H3	Oxidizing	H2
Highly flammable	H3-A	Flammable	H3-B
Irritant	H4	Harmful	H5
Toxic	H6 (including very toxic substances and preparations)		
Carcinogenic	**H7**	**Mutagenic**	**H11**
Corrosive	H8		
Infectious	H9		
Teratogenic	H10		

Substances or preparations which:

h) give off a toxic or very toxic gas on contact with water, air or an acid: H12
i) may give rise to a hazardous product after disposal: H13
j) are ecotoxic: H14

Council Decision 94/904 defining and completing the list of wastes which are hazardous by virtue of their concentration of hazardous substances and of their origin.

Decree 92-1261 of 3 December 1992 on the prevention of chemical hazard.

This concerns *inter alia* the classification of products which are carcinogenic, mutagenic and teratogenic.

Notice of 6 June 1970 setting the conditions for disposal of radioactive wastes, in particular limits for specific and total activity with reference to the groups of radiotoxicity defined in Decree 86-1103 of 2 October 1986.

Decree 94-609 of 13 July 1994 on the disposal of packaging waste.

Order of 12 December 1994 on the carriage of hazardous substances.

This sets out conditions and measures for the carriage of hazardous substances by road.

Order of 5 January 1993 on the preparation and forwarding of the mandatory safety information sheet giving details of storage and disposal procedures.

2. COLLECTION OF WASTES CONTAMINATED BY CARCINOGENIC/MUTAGENIC SUBSTANCES

Carcinogenic wastes generated during laboratory experiments, animal experiments or during the preparation of chemotherapy treatments must be collected separately from other wastes. These include solid wastes (contaminated single-use equipment, containers, syringes, bedding, gloves, protective coverings, animal cadavers, etc.) and liquid wastes (aqueous, organic, oily solutions). These wastes must be sorted according to the type of processing they will undergo.

Generally speaking, the criteria for sorting and conditioning wastes will be determined jointly with the company responsible for their collection, removal and final destruction in accordance with current legislation.

2.1 Solid wastes other than those from animal experiments

Solid wastes such as paper, filters, gloves, disposable protective clothing, masks, single-use small equipment, etc. must be placed in closed containers suitable for incineration. Hermetically sealed containers which are robust and can be incinerated must be used for this type of waste. Sacks must be no more than two-thirds full so that they can be properly closed by tying or sticky tape, to prevent spillage during their removal for destruction.

If you have an incinerator *in situ* which meets the required standards (Circular of 21 March 1983 and Order of 23 August 1989) you may carry out conditioning operations at your facility:

- of paper sacks suitable for incineration, with a capacity of 25 or 50 litres. Capacity will depend on the size of the incinerator's loading orifice. These sacks must be made of a double outer layer of kraft paper and an inner sheath of polythene.
- of waste drums whose capacity is smaller than that of sacks (0.5 to 7 litres). These are designed to stand on the work surface as they are filled, to avoid any spread of contamination. A range of models is available commercially. They are wide-

mouthed and are closed by a lid, sealing the receptacle so that the contents cannot escape if accidentally dropped.

Another type of container has been specially designed for the collection of different models of sharps (needles, sheaths). This enables needles to be safely discarded and stored. An automatic locking mechanism prevents any over-filling or escape of needles.

All containers must be labelled as follows:

Danger! Chemical carcinogens
solid waste

2.2 Liquid wastes

After handling, liquid wastes must be treated *in situ* by appropriate chemical methods or collected in small-volume bottles (1 to 2.5 litres). Receptacles for this, the first stage in collecting these wastes, must be placed on the work surface, preferably under a ventilated hood (cf. Chapter IV), as close as possible to the point where the work is carried out. They must be of plastic-coated glass, that is to say proof against the solvent or the carcinogen, and shock-proof. In no event should bottles made of polythene or another plastic or of metal, e.g. aluminium, which could be attacked by products, be used for this purpose. In the case of exclusively aqueous waste solutions, however, tough plastic bottles may be used.

Liquid wastes must be divided into three categories:

- aqueous liquid wastes, in which case the bottle must be marked:

Danger! Chemical carcinogens
liquid waste

- organic liquid wastes, in which case the bottle must be marked:

Danger! Chemical carcinogens
organic liquid waste

- oily liquid wastes must by law be separated from organic wastes. These must be stored in bottles marked:

Danger! Chemical carcinogens
oily waste

Unless a method of on-the-spot degradation has been chosen, which is most suitable for processing small quantities of waste, full bench-top receptacles must be collected and grouped, depending on category and their physicochemical compatibility, in 10, 20 or 30-litre containers suitably labelled, fully leak-proof and hermetically sealed. These are

supplied and removed by specialist companies (approved vehicles, trained personnel) responsible for transporting them to appropriate disposal facilities.

2.3 Culture media

- Cell culture media and bacterial suspension cultures: culture media and first washing liquids must be collected in bottles suitable for incineration, to which sodium azide is added (NB: not sodium hypochlorite, since this has been shown to intensify the mutagenicity of certain products).

  The bottles are treated as carcinogenic waste and incinerated. The conditions of incineration must be reviewed and agreed.

- Petri dishes containing agarose: all dishes which have been treated with a product shown to be mutagenic in one of the tests, including dishes containing insensitive strains, must be treated as carcinogenic waste.

2.4 Wastes from animal experiments

In addition to waste, the collection of which has been described above, when a dose of a carcinogen is given to an animal, this carcinogen or its active metabolites are likely to be found in the animal's excreta, contaminating bedding and cages. These cages must be marked:

Danger! Chemical carcinogens

- soiled bedding must be collected in tough sealed containers (plastic-lined purpose-designed cartons or double-thickness plastic sacks) which are labelled. These wastes are then treated in the same way as solid wastes (see relevant section). In most cases the choice will be incineration, because chemical methods, which have only been validated for a small number of cases, are not usually practicable for very large volumes of waste.

- cages must be decontaminated at once using a solvent in which the carcinogen is soluble, and rinsed before being sent for normal washing. The contaminated solvent must be disposed of as organic or aqueous carcinogenic liquid waste. In certain specific cases, cages may be decontaminated directly using suitable methods of chemical destruction.

- animal cadavers are placed in double-thickness plastic sacks, stored in an ice chest at -20°C and then treated as solid biological wastes to be incinerated in accordance with the legal rules for biological hazard.

2.5 Biological waste

Circular of 9 August 1978 concerning the Public Health Code: revision of model health regulations for the départements.

There are two categories of waste: contaminated and "specific" (Articles 86, 87 and 88).

This is currently the only reference text on infected wastes and states that the following wastes must be regarded as contaminated: "anatomical wastes, animal cadavers, putrescible excreta, any object, foodstuffs, soiled materials or culture media which may contain pathogens, together with single-use objects, plasters, liquid products and autopsy wastes."

Contaminated wastes must be incinerated.

Circular of 26 July 1991 on the implementation of procedures for disinfecting contaminated wastes from hospitals and equivalent establishments.

Prefectoral exemptions on the basis of an opinion delivered by the Supreme Health Council. Procedures allowing exemptions from this obligation, subject to interim treatment (**see next page**).

Law 92-654 of 13 July 1992 and implementing decrees of 27 March 1993 on the handling of and research on genetically modified organisms.

Decree 94-352 of 3 May 1994 on the prevention of biological hazard.

It is expected that this text will be followed by implementing decrees from the Council of State, particularly concerning the disposal of wastes contaminated by a microorganism.

The work of the European working group suggests that infectious wastes, currently known as "medical care wastes" may be divided into two categories:

- **non-hazardous wastes**, which entail no biological hazard but a psychological hazard deriving from the way in which the waste is perceived. These may contain disposable equipment or non-contaminated bedding. They are **equivalent to household waste** and are disposed of in the same way: collection either by the municipal refuse department or by a specialist waste company. They are either incinerated or held in storage (up to 2002).
- **Potentially infectious wastes,** comprising:
  - wastes contaminated by a microorganism of class 2, 3 or 4;
  - sharps;
  - anatomical items;
  - animal cadavers.

These must be collected in tough airtight, hermetically sealed receptacles made of polypropylene and marked with a pictogram indicating "biological hazard".

Length and conditions of storage are currently being studied. It is reasonable to assume that the storage time will be longer than 48 hours and that premises will have to meet certain criteria in respect of ventilation, temperature and decontamination facilities. They will have to carry warning notices.

Transportation must be in suitable vehicles with a separate container body which can be decontaminated.

Processing must take place in an approved incineration centre (specific centre or one in which medical care wastes make up 10% of all the waste it accepts).

Incineration is not the only type of processing. Under prefectoral exemptions, contaminated wastes (apart from anatomical items and animal cadavers) may be rendered harmless and then combined with equivalent household waste for further processing. There are currently 6 pre-treatment systems approved by France's Supreme Public Health Council and they apply to bulk wastes. They entail prior crushing in association with physicochemical decontamination methods (temperature, steam, microwaves, chemical methods, etc.). Others are being considered, particularly for small volumes. Before considering the use of interim treatment, one must check whether the procedure is compatible with the carcinogenic agent present in the waste.

2.6 Radioactive carcinogenic wastes

These must be marked:

Danger! Carcinogenic waste
and
radioactive products

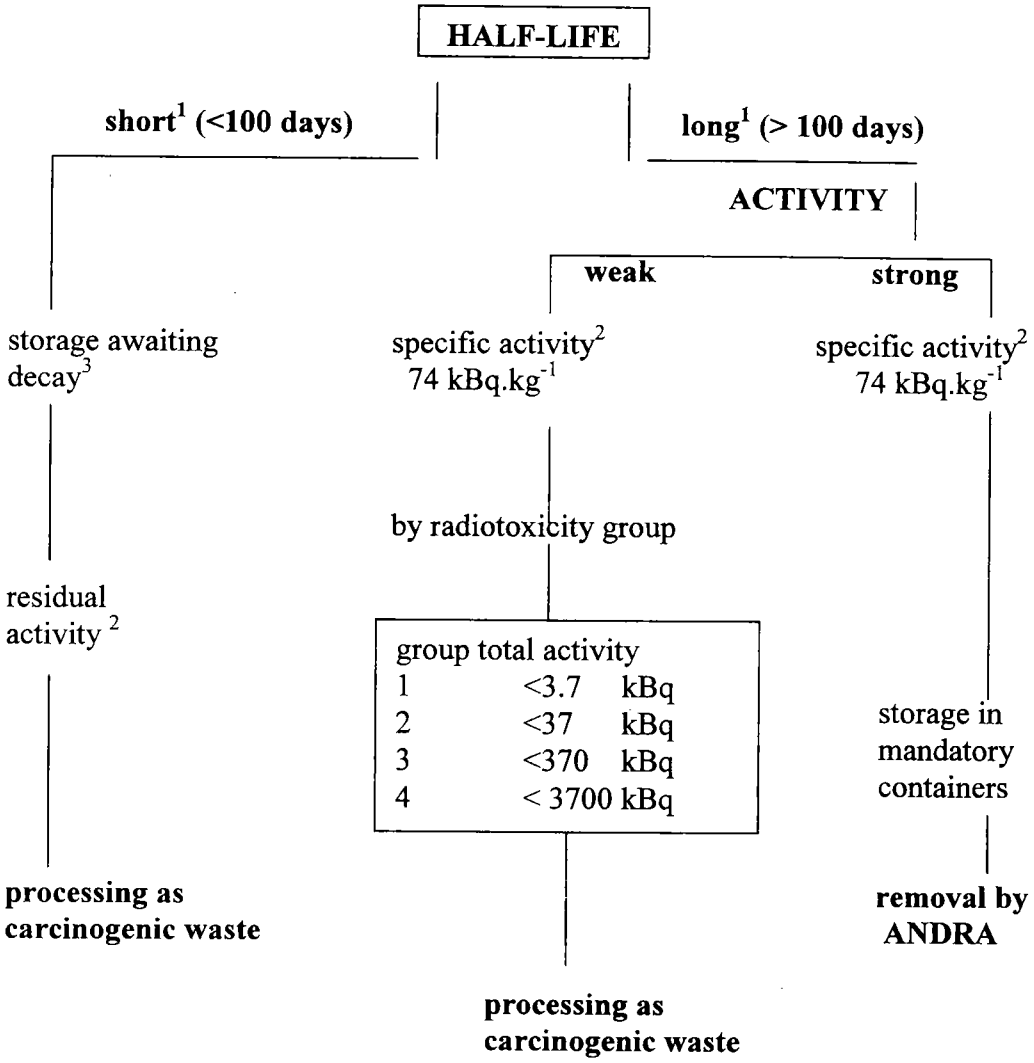
The containers used must be of the same type as those already described (cf. 2.1 to 2.3) provided this is compatible with the radiation emitted and they meet the obligations imposed by France's National Radioactive Waste Agency (ANDRA).

The processing of radioactive carcinogenic wastes will depend on their half-life and radiotoxicity (see Fig. 6).

3. USE OF INCINERATION

Incineration appears to be the method most commonly used at present to eliminate products contaminated by carcinogenic/mutagenic substances or known to be toxic. There is, however, a wide range of incinerators and their performance varies very widely. The efficiency even of those which perform best is so closely bound up with the conditions in which they are used (temperature, residence time, charging volume, combustion with or without post-combustion, etc.) that it is difficult to lay down strict rules to ensure that the final residues are non-hazardous.

Fig. 6. Radioactive carcinogenic waste



[1] Notice of 6 June 1970 (*Journal Officiel* of 6 June 1970)
[2] By day and building
[3] Before any decision to store labelled carcinogens is taken, the merits of doing so should be considered case by case.

Unfortunately materials contaminated with genotoxicants, bedding in particular, are processed in incinerators which are still in use in some hospitals or other establishments even though they do not meet the legislative requirements for incineration: minimum temperature 850°C for at least two seconds, adequate oxygen supply, limited fume emission, less than 3% of incompletely combusted materials (Circular of 21 March 1983 and Order of 23 August 1989), and do not provide satisfactory combustion. These practices are reprehensible and must be stopped, and the producer of the waste must ensure that it is disposed of properly. At present, the generally accepted scientific wisdom is that incineration with subsequent burning-off of gases at 1000°C for three seconds enables complex organic products to be efficiently destroyed with no emission of toxic by-products.

In the case of polyhalogenated, and especially polychlorinated products (PCBs), in order to prevent the emission of polychlorinated dibenzofurans and polychlorinated dibenzodioxins, newly formed and not destroyed, it is necessary to carry out post-combustion with the gases heated to 1200-1300°C for at least two seconds. When this is done, incineration of PCBs produces a PCB level of less than 50 nanograms per m^3 in fume and quantities which are also on the nanogram scale in solid residues.

Using these procedures the destruction yield is 99.9999% (Maes, 1991).

Highly efficient combustion of organic compounds is also achieved by incinerating them in converted cement kilns where the flame temperature is around 2000°C. This technique has even been recommended for the degradation of PCBs by the Canadian Government (MacDonald et al., 1977).

It is recommended that steps be taken to ensure that this treatment will be properly carried out by the company which takes charge of genotoxic waste for incineration. Whatever the centre at which final destruction is carried out, one has to bear in mind that the waste from research laboratories (which is small in quantity compared with industrial waste) will be reconditioned and incinerated in combination with other products.

A review by Castegnaro & Rousselin (1994) examines work published to date in the area of products used in research laboratories. Very few studies have been conducted on the degradation of carcinogenic substances using this method and the harmlessness of the residues does not appear to have been tested. It is in fact difficult to show that degradation is complete. It is worth mentioning the work of the NCTR (National Center for Toxicological Research) which shows that it requires 737°C for complete degradation of 2-acetylaminofluorene (Barbeito, 1979); that of Rubey et al. (1983) which shows that even at 725°C polycyclic aromatic hydrocarbons are only partly degraded; that of Rappe (1978) showing that dioxins are fairly stable up to 800°C and that at lower temperatures of about 300-500°C they can even form from precursors. A study in the USA of 18 incinerators given the same standard charge of non-carcinogenic products revealed that only three of them produced a satisfactory degree of degradation.

Regarding drugs used especially in hospitals, but also in laboratories, Wilson carried out a survey in 1981 of product manufacturers to draw up recommendations for the degradation of anticancer drugs (Wilson, 1983). Data on incineration are listed in Table 4, which also includes data published recently by Lee (1988) and Allwood & Wright (1993).

Table 4 prompts us to make the following observations:

- most of the instructions are incomplete because incineration depends on a number of factors, two of which are complementary - temperature and residence time;
- the information from 1983 and 1988 was far vaguer because the temperature was often not specified or reference was simply made to "normal conditions of incinerator use in the hospital environment", when at that time most incineration equipment operated at a low temperature (less than 800°C).

Growth in awareness of the risk between 1983 and 1993 resulted in more precise information and, in a number of cases, higher recommended operating temperatures.

Earlier work would seem to suggest that a temperature of 1000 to 1100°C in an incinerator with a twin-hearth furnace (combustion and fume recombustion) would be enough to degrade most substances. Even so, no studies seem to have been published to date on tests used to check that the fumes are indeed harmless. Industrialists, who have agreed to provide information, notably in the case of pharmaceuticals, should disclose the basis for the claims they make.

Two points should be noted which argue against the use of incinerators:

a) Because there are few high-performance incinerators, wastes have to be transported to the point of incineration. This creates an added risk because of the operations of truck loading and unloading (though a strict system – aimed to minimize handling – can be used whereby the containers are stored in crates which can be loaded without direct human handling) and because of risks of accident during transportation.

b) Incineration may be suitable as a means of processing waste, but it in no way directly resolves the problem of accidental contamination of surfaces (floors, work surfaces, etc.) and re-usable laboratory equipment (glassware, spatulas, etc.).

Accidental surface contamination can be dealt with by recovering the contaminant by means of a solvent, which can be collected on an absorbent product suitable for incineration, or by means of chemical treatment. Re-usable equipment can be decontaminated using a suitable solvent which will be treated as genotoxic waste or by a chemical destruction method which is also suitable for processing small quantities of waste.

Table 4. Use of incineration to degrade anticancer drugs

Compound	Processing method and temperature
Bleomycin	No temperature stipulated; hospital incineration conditions are described as adequate[1]; 1000°C[3]
Cisplatin	Temperature > 250°C, product must be carbonized[1], 250°C[2]; 800°C[3]
Cyclophosphamide	No temperature stipulated[1]; 900°C[3]
Dacarbazine	Same temperature as for solid wastes[1]; 500°C[3]
Doxorubicin	Temperature > 700°C[1]; 700°C[2-3]
Daunomycin	800°C[1-2]; 700°C[3]
Fosfestrol	No temperature stipulated[1]
5-fluorouracil	Pre-treatment followed by incineration at 1200°C for 2 seconds[1]; 1000°C[2]; 700°C[3]
Methotrexate	Normal conditions of incinerator use in a hospital environment[1], 1000°C[2-3]
Mithramycin	1000°C for at least 5 min.[1], 300°C[2]
Nandrolone decanoate or phenylpropionate	Normal conditions of incinerator use in a hospital environment[1-2]
Vincristine and vinblastine	No temperature stipulated[1]. 1000°C[2-3]
Vindesine	1000°C[2-3]
Aclarubicin	1000°C[3]
Amsacrine	> 260°C[3], 260°C[2]
Carboplatin	1000°C[3]
Carmustine	1000°C[2-3]
Cytarabine	1000°C[3]
Actinomycin D	1000°C[3]
Epirubicin	700°C[2-3]
Etoposide	1000°C[2], 700°C[3]
Idarubicin	700°C[2-3]
Ifosfamide	Normal conditions of incinerator use in a hospital environment[2]; 1000°C[3]

Table 4 (contd).

Compound	Processing method and temperature
Melphalan	500°C[3]
Mitomycin C	Normal conditions of incinerator use in a hospital environment[2]; 1000°C[3]
Mitozantrone	800°C[3]
Mustine	Incineration not recommended[2]; 800°C[3]
Plicamycin	1000°C
Thiotepa	800°C

References: Wilson, 1983[1]; Lee, 1988[2]; Allwood & Wright, 1993[3]

4. CHEMICAL DEGRADATION OF CARCINOGENIC/MUTAGENIC SUBSTANCES

A programme on this was launched in 1979 by the International Agency for Research on Cancer and the safety arm of the US National Institute of Health. First studies looked at the degradation of a number of families of genotoxic compounds and it became apparent, from the very first study (IARC, 1980; Castegnaro et al., 1981) that most studies on this subject had simply investigated the behaviour of the original product and had not studied its ultimate fate.

Consequently, the result of degradation in this study was either:
- in one case, the formation of a highly mutagenic product or,
- in the other cases, a reversible reaction which regenerated the initial product.

Similar problems emerged from evaluation of the methods published for the other families of carcinogenic compounds (Castegnaro & Rousselin, 1994).

When this programme was launched the Committee of Experts for the programme decided that the following points should be checked before a degradation method could be accepted (IARC, 1979):

- study of how efficiently the product was degraded, using the most sensitive analytical method possible;
- identification of absence of direct or indirect mutagenicity using the Ames assay on at least three different strains of *Salmonella typhimurium*;
- study of the method's reproducibility by organizing inter-laboratory tests.

The following criteria were also adopted to ensure that methods were acceptable for laboratory use:

- the method had to use routine, low-cost reagents;
- it had to be quick to perform.

On the basis of these criteria, ten volumes were prepared by IARC together with numerous publications by the various groups taking part in this study. They cover aflatoxins and other mycotoxins (IARC, 1980; Castegnaro et al., 1981; IARC, 1991a; Fremy et al., 1994), nitrosamines (Lunn et al., 1981; IARC, 1982; Castegnaro et al., 1982; Lunn et al., 1983b), nitrosamides (IARC, 1984a; Lunn et al., 1984; Lunn & Sansone, 1988), polycyclic aromatic hydrocarbons (Castegnaro et al., 1983; IARC, 1983a), polycyclic heterocyclic hydrocarbons (IARC, 1991b; Lunn et al., 1994), hydrazines (IARC, 1983b; Lunn et al., 1983a), haloethers (Alvarez & Rosen, 1976; IARC, 1984b), aromatic amines (Klibanov & Morris, 1981; IARC, 1985a; Lunn & Sansone, 1991a; Castegnaro et al., 1985), a series of anticancer and other drugs (IARC, 1985b; Barek et al., 1987; Lunn & Sansone, 1987b; Lunn et al., 1989), ethidium bromide (Quillardet et al., 1987; Lunn & Sansone, 1987a; Quillardet & Hoffnung, 1988; Bensaude, 1988; Muranyi-Kovacs, 1988), dimethyl and diethyl sulphates and methyl and ethyl methanesulphonates (Lunn and Sansone, 1985; De Méo et al., 1990; Lunn & Sansone, 1990), chromium^{+6} (Lunn & Sansone, 1989) and a series of halogenated compounds (Lunn & Sansone, 1991).

These methods are listed in Table 5 which also indicates their field of applicability. Before any of them is used, however, the original publications indicated above should be consulted.

As we can see from this Table, there is no universal method for degrading products with genotoxic potential. However, three methods are suitable for a wide range of products: oxidation using potassium permanganate in an acid or alkaline medium and reduction using a 50/50 mix of nickel/powdered aluminium in an alkaline medium. One can only encourage research by other teams too, so that the feasibility of using these methods in other areas can be studied more fully.

It must be emphasized, however, that the chemical products used in these techniques are highly reactive and may present a risk of violent reactions. Techniques of chemical destruction should only be employed where every safety precaution has been duly taken: adequate scientific equipment, appropriate and well-maintained collective and individual protective equipment, expertise and the time to apply it.

Table 5. Applicability of methods studied

DEGRADATION METHOD USED	PRODUCT FAMILY/PRODUCT STUDIED	VALIDITY	NOTES
Sodium hypochlorite	Aflatoxin B1	-	Mutagenic residues
	Citrinin	+	
	Ochratoxin A	+	
	Nitrosamine	-	No degradation
	PAH	-	Degradation variable
	1,1-dimethylhydrazine	+	Formation of N-nitrosodimethylamine
	3,3'-diaminobenzidine	-	Mutagenic residues
	Doxorubicin	-	Mutagenic residues
	Daunorubicin	-	Mutagenic residues
	Methotrexate	+	
	Ethidium bromide	-	Mutagenic residues
Calcium hypochlorite	Hydrazines (12 h)	+	Shorter times give mutagenic derivatives
Sodium hypochlorite then acetone	Aflatoxins	+	
	Sterigmatocystine	+	
Hot ammonia	Aflatoxins	+	
	Citrinin	+	
	Ochratoxin A	-	
	Patulin	+	
Cold ammonia	Haloethers	+	
Potassium hydroxide	Halogenated compounds	+	17 compounds studied
NaOH	Aflatoxins	-	
	Nitrosamides	-	Formation of diazoalkane
Sodium bicarbonate	Nitrosamides	+	
Sodium carbonate	Dimethyl sulphate	+	
Ammonium hydroxide	Dimethyl sulphate	+	
Alkaline hydrolysis in presence of hot DMF	Cyclophosphamide	+	
	Ifosfamide	+	
$KmnO_4$/NaOH	Aflatoxins	+	
	Citrinin	+	
	Ochratoxin A	+	
	Patulin	+	
	Sterigmatocystine	+	
	PHH	+	Except for dibenz(a,h)acridine

Table 5 (continued)

DEGRADATION METHOD USED	PRODUCT FAMILY/PRODUCT STUDIED	VALIDITY	NOTES
Ni-Al in an alkaline medium	Nitrosamines	+	
	PHH	-	Lack of reproducibility
	Hydrazines	+	
	4-nitrobiphenyl	-	Formation of 4-aminobiphenyl
	2-aminoanthracene	-	Incomplete reaction
	Azoic compounds	+	Except for phenylazoaniline, 4-dimethylaminoazobenzene, 4-amino-2,3-dimethylazobenzene (mutagenic residues)
	Isoniazid	+	
	Iproniazid	+	
	Ethidium bromide	-	Mutagenic residues
	Halogenated compounds	+	31 compounds tested
Sulphuric acid	Sterigmatocystine	-	Mutagenic residues
	HPA	+	
	Dibenzo(c,g) and (a,i) carbazole	+	
	Dibenz(a,h) and (a,j) acridine	-	
	Nitrosamides	-	
Sulphuric/chromic acid mixture	Aflatoxins	-	Formation of $Cr+^{6}$
	Nitrosamines	-	Formation of $Cr+^{6}$
	PAH	-	Formation of $Cr+^{6}$
Diazotation in presence of sulphuric acid	Aromatic amines	+	Except for 3,3'-dichlorobenzidine, 3,3'-dimethyloxybenzidine and 1-naphthylamine (mutagenic residues)
Powdered zinc/H_2SO_4	Cisplatin	+	
Powdered zinc/H_2SO_4 then $KMnO_4$	4-nitrobiphenyl	+	
$KMnO_4/H_2SO_4$	Aflatoxins	+	
	Nitrosamines	+	
	PAH	+	
	PHH	+	
	Nitrosamides (30 min)	-	Mutagenic residues
	Nitrosamides (8 h)	+	

Table 5 (continued)

DEGRADATION METHOD USED	PRODUCT FAMILY/PRODUCT STUDIED	VALIDITY	NOTES
$KMnO_4/H_2SO_4$	Hydrazines (8 h)	+	Except for dialkyl- and diaryl- which give the corresponding N-nitrosamines, and in the presence of DMSO
	Aromatic amines	+	
	Azoic compounds	+	Except for azoxybenzene (incomplete degradation) and 4-dimethylaminoazobenzene (mutagenic residues)
	2-aminoanthracene	+	
	Cyclophosphamide	-	Mutagenic residues
	Ifosfamide	-	Mutagenic residues
	Cisplatin	-	Mutagenic residues
	Melphalan	-	Mutagenic residues
	Lomustine	-	Mutagenic residues
	Chlorozotocin	-	Mutagenic residues
	Dacarbazine	-	Mutagenic residues
	Doxorubicin	+/-	
	Daunorubicin	+	
	Methotrexate	+	
	Dichloromethotrexate	+	
	Vincristine sulphate	+	
	Vinblastine sulphate	+	
	6-thioguanine	+	
	6-mercaptopurin	+	
	Streptozotocin	+	
Copper (I) chloride/HCl	Nitrosamines	-	Lack of reproducibility
Sulphamic acid/HCl	Nitrosamides	+	Except for N-nitroso-N-ethylurethane
Powdered iron/HCl	Nitrosamides	+	Except for N-nitroso-N-ethylurethane, N-methyl-N'-nitro-N-nitrosoguanidine, N-ethyl-N'-nitro-N-nitrosoguanidine and in the presence of acetone
Potassium iodate/HCl	Hydrazine	+/-	

Table 5 (continued)

DEGRADATION METHOD USED	PRODUCT FAMILY/PRODUCT STUDIED	VALIDITY	NOTES
HBr in glacial CH_3COOH	Nitrosamines	+	Except aqueous medium and DMSO
	Nitrosamides	+	Except N-nitroso-N-methylurea
	Carmustine	-	Mutagenic residues
	Semustine	-	Mutagenic residues
	PCNU	-	Mutagenic residues
	Lomustine	+	
	Chlorozotocin	+	
	Streptozotocin	+	
Acid hydrolysis in presence of HCl, addition of Na_2SO_4 then alkaline hydrolysis	Cyclophosphamide	+	
Peroxotrifluoro-acetic acid	Nitrosamines	-	Mutagenic residues
Diazotation in presence of phosphinic acid	Aromatic amines	+	Except 1- and 2-naphthylamine and 2,4-diaminotoluene (mutagenic derivatives) and 3,3'-diaminobenzidine (incomplete reaction)
	Ethidium bromide	+	
Precipitation by H_2O_2/peroxidase	Aromatic amines	+	Except for 4-aminobiphenyl. Solid residues must undergo another treatment.
Sodium phenate	Haloethers	+	
Sodium methoxide	Haloethers	+	
Sodium diethyldithio-carbamate	Cisplatin	+	
Sodium thiosulphate	Dimethyl and diethyl sulphate Methyl or ethyl methanesulphonate	+ +	
Sodium metabisulphite then magnesium hydroxide	Cr^6	+	

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XI.

PROCEDURE TO BE FOLLOWED IN THE EVENT OF ACCIDENTAL CONTAMINATION

N. WEILL

PROCEDURE TO BE FOLLOWED IN THE EVENT OF ACCIDENTAL CONTAMINATION

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This type of accident must be systematically planned for because it can affect the entire staff of a laboratory and its equipment. The genotoxic substance in question may arise in a number of forms (liquid, solid, gas, volatile product, aerosol, etc.) and every eventuality must be catered for.

In every case:
- emergency exits must be signposted, telephone numbers must be displayed (poison control centre, fire brigade, ambulance service, medical centre);
- these emergency services must be notified of the existence of the hazard and of the proposed protocol;
- emergency equipment must be to hand and trained first-aiders must be available.

1. IMMEDIATE ACTION

In every case:
Persons whose names and telephone numbers are clearly displayed on the door to the premises concerned must be informed.

It is the responsibility of the supervisor to notify the medical service which must record the accident in the register of accidents at work and who will contact outside services if necessary together with the health and safety committee/works council.

The immediate action taken by the supervisor has a number of objectives:
- to evacuate personnel quickly in accordance with a pre-arranged plan if the contamination is due to a gas, volatile product, aerosols, powdery solids or liquids;
- to avoid air currents: doors must be closed, hoods switched off if the contaminant is a powder;
- to take preventive measures to restrict access to the contaminated area;
- to organize prompt decontamination of exposed personnel using appropriate methods;
- to organize prompt decontamination of the premises and equipment exposed.

Adequate precautions must be taken to prevent contamination of premises, equipment and individuals as far as possible.

2. EVACUATION OF PERSONNEL

This must be done very promptly in serious cases of major contamination and where the product may easily disperse (as in the case of gases, volatiles or aerosols). This evacuation may require the assistance of persons from outside wearing protective clothing which is appropriate to the scale and type of contamination (gloves, goggles, cellulose mask, cartridge mask, self-contained breathing apparatus, combination).

3. DECONTAMINATION OF PERSONNEL

Any signs of acute intoxication and/or a life-threatening condition (injuries, breathing difficulties) must be attended to first.

After that, and depending on the type of contamination, there are a number of possible scenarios: in every case, clothing which has been soiled or is thought to have been soiled must be removed for decontamination and placed in special sacks.

3.1 Contamination of the skin and mucosa

Copious and immediate washing must be carried out on the spot for 20 minutes using cold or tepid water delivered by a shower, eye bath, or any other suitable method.

Never rub or scrub and never use a solvent, including alcohol, which speeds up passage through the skin.

The contaminant is diluted by this first rinsing, and the water must be discarded together with mutagenic waste.

If the suspect products are lipophilic (solubility in water < 0.1%) mild detergents may be used on the skin to complete the decontamination.

Use of detergents, however, must remain the exception, because it can make it easier for the contaminant to penetrate the skin or mucosa and it should never be used as the method of first resort.

In severe cases, this contamination may be continued in hospital where any systemic effects can be treated.

3.2 Absorption by mouth

The process of decontamination follows medical or hospital practice (poison control centres). The mouth may, however, be rinsed out on the spot if the individual is conscious. **Never induce vomiting in an accident victim.**

3.3 Inhalation

Apart from evacuating the subject immediately to a non-contaminated area, the process of decontamination will require the attention of specialist personnel. The subject may need to be taken to hospital for the toxic effects of the contaminant to be treated.

4. DECONTAMINATION OF PREMISES AND EQUIPMENT

In every case:
- The safety service must be notified;
- The contaminated area (floors, bench tops, etc.) must be marked off and isolated using a marker or sticky tape;
- Appropriate protective clothing must be put on (gloves, cellulose mask or cartridge mask or self-contained breathing apparatus, combination);
- Nothing must be picked up with the bare hands;
- Decontamination must be carried out.

Where the contaminant is a liquid:

Absorbent products (e.g., Labosi universal drying agent) may be spread over the soiled surfaces. These absorbent products must then be disposed of in receptacles set aside for genetic toxicants. This area should then be copiously washed and rinsed using a solvent appropriate to the contaminant and disposing of the rinsing and washing liquids as mutagenic effluent. The final rinsing liquid should be tested for mutagenicity, a chemical analysis should be conducted, which is faster than the mutagenicity test, and access to the contaminated area must be prohibited until such time as the test results are known. **In every case, solutions must be wiped up working from the outside edge of the soiled area in towards the point of first impact to prevent the hazardous product from spreading.** These operations must be performed by a properly protected competent individual.

Where the contaminant is a powder:

All forms of ventilation must be switched off to reduce the risk of dispersion and the contaminated area must be cleaned using paper or a cloth impregnated with solvent. Filters must be changed after decontamination. The contaminated area must be covered by a cloth or compresses soaked in water or a neutralizing solution to prevent the generation of respirable particulates.

Premises and equipment may also be decontaminated using a wet method, i.e. initially use of specific solvents, decontaminants or detergents in an aqueous solution or soapy water. These solvents, decontaminants or detergents should be spread on absorbent paper and discarded after use in the receptacles for toxic substances. Surfaces should be copiously rinsed before the premises are used again.

In every case, **solutions must be wiped up working from the outside edge of the soiled area in towards the point of first impact to prevent the hazardous product from spreading.**

Small equipment may be disposed of without cleaning given its low cost; alternatively it should be decontaminated using the same method as above.

Clothing contaminated by accident or used during cleaning must be incinerated.

5. EMERGENCY STAND-BY EQUIPMENT

An eye bath and a shower should be close to hand near a laboratory which uses mutagenic products.

A stock of latex gloves, cellulose masks, self-contained breathing apparatus, combinations, paper, disposable hooded coats, overshoes and drying agent must be made available to personnel. Ideally, a special spill control kit containing the various items of equipment needed should be assembled.

Lastly, it is vital to have a telephone in the immediate vicinity next to which the telephone numbers of the supervisor, the medical service, fire brigade, ambulance service, poison control centre, etc. are displayed. This must be located in the corridors and not in the laboratory itself.

6. ACTS OF VANDALISM, THEFT, FIRE, FLOOD

Procedures must be geared above all to prevention. However, the laboratory supervisor must be notified at once of any incident so that appropriate action can be taken. Information must be given at once to emergency teams which may have to be brought in from outside. All personnel must be made aware of any theft or act of vandalism, by posting up the details so that everyone is informed of the hazards entailed by the product.

7. NOTIFICATION OF ACCIDENTAL CONTAMINATION

This must be done using a standard-format document. A copy of it must in every case be sent to the medical service.

The document must state:
- the day and date of the accident;
- the names of the persons concerned, including those who helped in the decontamination work;
- the premises and equipment contaminated;
- the name of the product which has accidentally been dispersed, together with its volume, presentation and concentration;

- a description of the operations which resulted in the accident;
- a description of the actions taken after the accident;
- all these details must be entered in the safety register (cf. Chapter XII).

8. RESPONSIBILITY

The laboratory supervisor has a duty to inform persons handling products which are known or suspected mutagens and/or carcinogens of the potential hazards.

Specific procedures must be available for personnel to follow.

In the event of an accident, a subsequent inquiry must determine the causes of the accident and ways of ensuring that recurrences can be prevented.

XII.

ORGANIZATION AND MANAGEMENT OF SAFETY IN A LABORATORY HANDLING GENOTOXIC AND CARCINOGENIC PRODUCTS

D. MARZIN

ORGANIZATION AND MANAGEMENT OF SAFETY IN A LABORATORY HANDLING GENOTOXIC AND CARCINOGENIC PRODUCTS

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Summary

The organization of safety in a laboratory handling genotoxic products ***must include a set of safety rules,*** *with a* ***programme for applying the rules*** *and a* ***procedure for appointing the safety officer.***

Responsibility for compliance with the safety rules rests with the head of the establishment or laboratory concerned.

A safety officer must be appointed; his duty is to ensure that the safety programmes and rules are implemented.

Every employee must notify the safety officer of any actions or conditions which are unsafe or any procedures whereby safety may be improved.

The safety officer must have a thorough grounding in problems of genotoxicity.

The safety officer must organize training for personnel.

1. DUTIES OF THE SAFETY OFFICER

The most important activities which the safety officer must perform include the following:

a) He must carry out periodic safety checks of methods and equipment, talk to and inform persons involved in infringements of the safety rules.

b) He must ensure that all personnel are adequately informed and thus aware of the hazards.

c) He must ensure that all personnel receive ongoing training in safety.

d) He must satisfy himself that any change to the safety rules is brought to the notice of all personnel concerned.

e) He must conduct an inquiry after every accident, even if no member of the laboratory staff was exposed or affected, and must submit his findings to the director and the health and safety committee.

f) He must ensure that decontamination procedures are properly carried out.

g) He must help to monitor sicknesses and absences of laboratory personnel which might possibly be due to occupational factors.

h) He must check that operators have decontaminated equipment after use and that the safety rules are observed in the disposal of waste.

i) He must take charge of decontamination operations and emergency measures in the event of accidental contamination.

j) He must oversee the keeping of an in-out file or book which records all movements of genotoxic products into and out of the laboratory and must ensure that any person working in the laboratory who introduces a potential genotoxicant declares the fact.

k) He must examine all experimental plans and protocols for their safety implications.

l) He must set up a duty roster to cover any emergencies occurring outside normal working hours.

m) He must designate a person to take over all or part of his safety officer's duties in his absence.

n) He must brief the health and safety committee on the nature of safety problems and the solutions adopted to resolve them.

o) He must keep an up-to-date safety register.

2. SAFETY REGISTER

a) Every laboratory handling genotoxic products must have a safety register.

b) The safety officer or his designated deputy is responsible for keeping the safety register.

c) The safety register must record:

   i) Any incident or accident occurring in the laboratory, whether or not it has been the subject of a report; for each of these incidents or accidents the following details must be given:
   . the date it occurred;
   . any persons exposed during or after the accident;

. individual and collective measures taken to decontaminate individuals, equipment and premises;
. persons notified of the accident (occupational physician, management, etc.).

ii) Actions or conditions which are identified as being unsafe, procedures designed to improve safety which the safety officer, his deputy or any other employee suggests. For each notification the following details must be recorded:
. the date;
. the form of notification (written, oral);
. the persons concerned (persons exposed and/or persons able to report);
. persons notified;
. measures taken to effect the requisite changes or reasons why such changes were not made;
. dates when changes were made.

Written notifications must be held in the safety register.

iii) Any changes to the safety rules and the reasons for them.

3. SAFETY RULES

a) Every laboratory handling genotoxic products must have a set of safety rules setting out the safety procedures to be followed.

b) The safety officer is responsible for drawing up the safety rules and making changes to them.

c) Safety procedures must include the following points at least:
. rules for appointing the safety officer and his deputy;
. role and duties of the safety officer;
. purchasing and storage of genotoxic products;
. handling of genotoxic products;
. rules for use of equipment;
. personal protection and medical surveillance of personnel;
. procedures to be observed in the event of accidental contamination;
. rules for marking and identifying premises;
. list of individuals authorized to handle products or who may be exposed;
. rules governing access to premises.

d) Any change to the safety rules must be acknowledged and dated by the safety officer. Changes to the safety rules and the reasons for them must be recorded in the safety register.

e) The safety rules and any change to the rules must be made known to the personnel concerned. The full text of the rules must be available at all times. In order to be sure that every person concerned is familiar with the rules or changes to them, each person concerned must date and sign these documents (the signed documents are retained and held by the safety officer).

4. AUTHORIZED PERSONNEL

a) Only authorized persons may have access to premises in which genotoxic products are handled. The list of those authorized to enter a controlled area must be recorded in the procedures laid down in the safety rules.

b) Persons may be granted temporary access to certain premises subject to the assent of the safety officer. In this case, the safety officer stipulates the premises to which they may have access and under what conditions (protective clothing to be worn, etc.).

The list of persons granted temporary access must be recorded in the safety register.

c) Personnel granted permanent or temporary access must undertake to be bound by all the safety rules.

5. TRAINING

The safeguards provided by the laboratory and specially designed equipment to protect personnel are powerless against human error and unsatisfactory practices. Consequently personnel must:
- be routinely reminded of the safety rules;
- know how to recognize and control hazards;
- receive ongoing training in safety.

Periodic training programmes for personnel must be organized in every laboratory which handles genotoxic products. The safety officer is responsible for implementing these.

Training programmes may, for example, cover the following points:

a) General

i) Sources, in the laboratory, of
. biological hazard
. chemical hazard
. physical hazard.

ii) Rights and obligations of the worker in regard to safety.

b) Precautions on entering

i) Access to the laboratory
ii) Personal hygiene
iii) Protective clothing.

c) Conduct of experiments

i) Use of pipetting systems
ii) Reduction of aerosol formation
iii) Correct use of safety enclosures
(laminar flow hoods, cabinets, incubators, etc.)
iv) Precautions to be taken during weighing.

d) Emergency procedures

i) First aid
ii) Liquid spillages, broken receptacles, local decontamination
iii) Accidents

e) General laboratory maintenance

i) Storage of hazardous materials
ii) Transportation of hazardous materials
iii) Cleaning of equipment and premises.

f) Precautions on leaving

i) Disposal of hazardous waste
ii) Decontamination methods
iii) Personal hygiene.

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