COMMISSION OF THE EUROPEAN COMMUNITIES

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R E P O R T

on the possibility of modifying tests and guidelines laid down in existing Community legislation in compliance with Article 23 of Council Directive 86/609/EEC on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes

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(presented by the Commission)

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1. Article 23 of Council Directive 86/609/EEC[1] on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes reads :

"Article 23

1. The Commission and Member States should encourage research into the development and validation of alternative techniques which could provide the same level of information as that obtained in experiments using animals but which involve fewer animals or which entail less painful procedures, and shall take such other steps as they consider appropriate to encourage research in this field. The Commission and Member States shall monitor trends in experimental methods.

2. The Commission shall report before the end of 1987 on the possibility of modifying tests and guidelines laid down in existing Community legislation taking into account the objectives referred to in paragraph 1."

2. As a background it is useful to clarify what is meant by a toxicity test and to describe some of test procedures which are commonly used; a summary of this information is given in Table 1.

3. While it is difficult to obtain precise information on animal experimentation throughout the Community, a conservative estimate of the total number of animals used each year would be around 10 million of which about 20 % are used for the purposes of satisfying regulatory requirements; of the remaining 80 % the majority are used either for academic research or

(1) O.J. No. L 358, 18.12.86, p. 1

industrial research and development. It follows that modifications to regulatory testing requirements can only have an impact for the 20 % of animals used for such purposes.

4. Several pieces of Community legislation explicitly or implicitly require animal testing to be carried out (see Table 2). A detailed description of the testing requirements allied to each piece of legislation can be found in EUR Report 11353 "EEC Directives and Animal Testing".

5. In the majority of cases, references to animal testing in Community legislation take the form of guidelines, or notes for guidance, concerning necessary toxicological data which should accompany an application for the placing on the market, or the use, of certain products or ingredients. In some instances such guidelines, or notes for guidance, form part of the text of the legislation itself, as is the case for pharmaceuticals (see Council Recommendations 83/571/EEC and 87/176/EEC), whereas for other product areas they have been elaborated by one of the Communities' Scientific Advisory Committees (see for example Report of the Scientific Advisory Committee for Food - "Guidelines for the Safety Assessment of Food Additives" - opinion of 22nd February 1980 contained in EUR Report 6892 or Report of the Scientific Committee on Cosmetology - "Notes of guidance for the toxicity testing of cosmetic ingredients" - opinion of 28 June 1982 contained in "EEC Environment and Quality of Life" 1983).

Where use is made of guidelines or notes for guidance the precise details of the testing methods to be used are not spelled out and readers are usually referred to appropriate internationally accepted testing methods. Frequently referred to sources for detailed testing methods are the guidelines for the testing of chemicals published by the Organization of Economic Co-operation and Development (Annex 1 to Decision of

OECD Council C(81)30 1981). The European Pharmacopoeia (elaborated under the auspices of the Council of Europe) and the Communities' own chemicals control legislation (Council Directive 67/548/EEC (see para 6 below)). The use of guidelines or notes for guidance often allows sufficient flexibility so that alternative methods (see para. 9) can be used as and when they become available.

6. Directive 67/548/EEC as amended for the sixth time by Directive 79/831/EEC concerns the classification, packaging and labelling of dangerous substances. The Directive requires that before a new substance can be placed on the market a technical dossier must be submitted containing, among other information, toxicological data, including LD50 values, and the results of eye and skin irritation tests. The methods which have to be followed in completing these tests are described in detail in Annex V of the Directive, the first part of which was published as a Commission Directive in 1984 (84/449/EEC) Other pieces of Community legislation, existing and proposed, refer to the testing methods required under Directive 67/548/EEC, e;g. additives in animal nutrition, Council Directive 87/153/EEC and the Commission proposal for a Council Directive concerning dangerous preparations (COM(85)364 final)[2].

Directive 67/548/EEC is a central piece of Community legislation which has harmonized chemicals control in the Community. However, it has frequently been critized as a piece of legislation entailing unnecessary testing.

In the face of such criticism it has to be pointed out that before its introduction each of the Communities' Member States could require different information to be supplied before substances were placed on the market and that this often led to a duplication of animal testing. Now, with the mutual acceptance of data, tests only need to be carried out once according to

(2) O.J. No. C 211, 22.08.85, p. 3

the prescribed method and the results are accepted throughout the Community. It is therefore undoubtadly the case that Directive 67/548/EEC has brought about a considerable reduction on the total number of animals used for chemicals testing. Secondly, the number of animals used for satisfying the requir[illegible]omical regulation testing probably represents less than 5% of the total number of animals used annually. The Commission is, however, sensitive to its responsibilities to renew the testing requirements of Directive 67/548/EEC and an updating procedure has already been initiated (see para.16).

7. The reason why toxicity testing is required to be carried out is to ensure adequate protection for man and/or other specific target species e.g. farm animals, and in some cases, additionally, the environment. The rationale has been that one carries out tests on certain animal species so that from the results one can extrapolate to the likely effect on man or other target species and thereby prevent or control the placing on the market of potentially dangerous products; implicit in such an approach is the acceptance that the animal species used in testing are appropriate models for the species one is trying to protect.

8. In recent years the testing schemes used for regulatory purposes have been the subject of criticism from the point of view of their scientific validity, and there has been mounting pressure to introduce methods using fewer or no vertebrate animals.

While the Commission is committed to the objective of actively seeking such alternative methods it considers that its primary responsibility lies in protecting man and the environment from the risks arising from the placing on the market of potentially dangerous products. Therefore, the Commission will only consider the acceptance of an alternative method when it has

been demonstrated that it affords the same level of security for the protection of man and the environment as the existing procedure(s) it is designed to replace (see para 12).

9. Before considering the opportunities for the amendment of current test guidelines it is useful to discuss what is meant by an alternative or modified method. Russell and Burch (1959)* proposed that alternatives could be considered under three headings reduction, refinement or replacement (the so called 3Rs).

- reduction alternatives, which reduce the number of animals required;
- refinement alternatives, which diminish the amount of pain and distress suffered by animals used in testing procedures;
- replacement alternatives, which completely replace animal experiments.

The principal types of technique which lead to reduction, refinement or replacement of animals tests are listed in Table 3.

10. At the present time the majority of proposed alternatives fall under the headings of refinement or reduction and although methods involving replacement by non sentient material or lower orders of organisms are available few, if any, have been developed to the stage where they could be proposed for acceptance within a regulatory testing system (Table 4). In addition, the development of new methods has to a large extent been concentrated on the identification of alternatives for the acute toxicity testing procedures e.g. the acute LD50 and Draize tests (see para 2).

(*) Rusell, WMS and Burch RL (1959) Principles of Human Experimental Techniques (Methuen London)

The opportunities available to the Commission for immediate action are consequently limited to those few alternative methods which have gained widespread acceptance.

11. There is considerable research activity within the Member States directed towards the development of alternative test methods. In addition, the 4th European Community Environmental Protection Research ann Development Programme (1986-1990) and The European Community Biotechnology Action Programme (1986-1989) both include research initiatives which should lead directly or indirectly towards the same goal. Furthermore, a number of studies have been financed within the framework of the European Communities Toxicology Action Programme with the objectives of reducing both animal usage and animal suffering and of promoting new approaches to toxicity testing, particularly in vitro methods.

12. There are many factors which govern the acceptability/utility of alternative test methods and any proposed test method must undergo rigorous evaluation before it can be accepted at a regulatory level. While the scientific merit can often be assessed, to a large degree, during the early stages of development (as part of a research programme) there comes a point when a method has to be evaluated against other criteria e.g. practicality, cost effectiveness, repeatability, comparison with methods it is designed to replace and comparison with other alternative methods. This wider evaluation often involves the organisation of "round robin" or "ring-test" exercises where a large number of laboratories try out the proposed test procedure using the same set of reference chemicals. These ring test exercises often highlight practicial problems; for what may be common place in one laboratory may present unexpected difficulties in another. It is essential that this wider evaluation of alternative

methods be undertaken at an international level : if a method is only accepted within one Member State this will have a negligible impact. The Commission is ideally placed to co-ordinate the evaluation of alternative methods at a Community level.

13. As alternative methods are developed it is important that potential users (researchers, industry, regulators) are aware of their availability and current status (early stages of development, being ring-tested, accepted for regulatory testing etc.). It is therefore important, both from the point of view of avoiding duplication of research effort as well as from the animal welfare perspective, that there is an efficient procedure for information exchange. Within the Community, the Commission is ideally placed to ensure the necessary co-ordination of this information exchange.

14. It is evident that one of the major obstacles towards the acceptance of methods to replace the classical LD50 testing procedure is the fact that many nations and international bodies use the results of such tests as a means of classifying chemicals (Table 5). Therefore, if the Community were to unilaterally adopt non-LD50 testing procedures accompanied by parallel changes in its classification scheme this would, in the absence of simular modifications by third countries, only result in Community manufacturers having to carry out the normal LD50 testing in order to export. It is therefore essential that any action on this question is undertaken at an international level and in a harmonized manner.

The Commmission's Proposals

15. From the foregoing it should be apparent that the immediate opportunities for modifying tests and guidelines in existing Community legislation are limited. The Commission's proposals are therefore predominantly concerned with an ongoing committment to the development of alternative methods and the creation of a framework wherein alternative methods can be introduced in the legislation as they become available.

16. The Council of the Organization for Economic Co-operation and Development adopted on 24 February 1987, updated versions of their testing guidelines 401, Acute Oral Toxicity; 402, Acute Dermal Toxicity; and 405, Acute Eye Irritation. The modifications introduced in these updated versions include the reduction of the numbers of animals used and the reduction in the degree of suffering experienced by the test animals. The Commission undertakes to introduce the necessary modifications to existing Community legislation. In practice this will mean the publication of a Commission Directive adapting to technical progress annex V of Directive 67/548/EEC (see para. 6);

17. The Commission will continue to be involved with, and to support, the OECD's updating programme with respect to the guidelines for testing of chemicals.

18. Pyrogenicity testing (testing for fever-inducing characteristics) is required in registering some pharmaceuticals. Pyrogenicity testing normally requires injecting rabbits with the potential pyrogen and observing any reactions to the substance. Extensive work has been done to establish and validate an alternative test for pyrogenicity

using the asiatic horseshoe crab (Limulus polyphemus). This test, called the limulus amoebocytelysate (LAL) test, uses an extract from the blood cells of the crab which can be used for in-vitro testing. As soon as this test method is introduced into the European Pharmacopoeia it will automatically be acceptable under the relevant Community pharmaceuticas legislation.

19. The Commission has already initiated an updating programme for Annex V of Council Directive 67/548/EEC relating to the classification, packaging and labelling of dangerous substances (see para.6). In addition to the proposals indicated under para 16 (above) the Commission, in consultation with the Member States, will also evaluate the possibility of introducing other alternatives to the current LD50 testing procedures as well as replacements for the current eye and skin irritation tests. As and when such alternative methods are accepted they will be introduced into Annex V of the Directive by the publication of a Commission Directive adapting the annex to technical progress.

20. The Commission proposes to hold an international symposium in early 1989 to explore the possibility of a phased replacement of classical toxicity testing methods carried out for the purposes of classifying dangerous chemicals.

21. The Commission will continue to fund research activities in the field of alternative test methods (see para 11). However, the Commission is aware of the considerable research activities taking place within the Member States, both publicly and privately funded, and proposes that the Commisison should be

involved in the co-ordination of information exchange on these research activities.

22. As part of this co-ordination effort the Commission would propose to fund the development of a European Data Base on Alternative Toxicity Testing Methods such that researchers, industry and governments could be immediately aware of the developments taking place throughout the Community; such activity to be undertaken in consultation with other international bodies e.g. W.H.O. and O.C.D.E. In this context the Commission has already established an inventory of in vitro testing facilities within the Community.

23. The Commission recognizes that a critical stage in the development of an alternative method is the transition from that of a potentially useful procedure to that of a method accepted as part of regulatory testing system (see para. 12). The Commission therefore proposes to provide a framework for the evaluation of alternative test procedures.

24. The Commission will make proposals for Community action in internationl fora, particularly in the Council of Europe and O.C.D.E., where questions of animal experimentation and alternative methods are discussed and will also seek to promote common action by the Member States, when appropriate, in areas covered by Member State competences.

In particular it is hoped that, dependent upon the outcome of the symposium referred to in paragraph 20, the Commission would be in a position to formulate a proposal concerning LD50 testing during the course of 1989.

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TABLE 1

TOXICITY TESTING

The objective of toxicity testing is to measure the toxicity of any substance in order to determine what doses are safe; to assess the types of injury which occur if safe levels are exceeded and thus to enable calculations of risk versus benefit and methods of safe handling to be drawn up.

Types of Toxicity

Acute toxicity comprises the effects found within a few days of a single dose and includes lethal effects. An LD_{50} value is the dose at which half the test animals can (within statistical limits of confidence) be expected to die. Exposure to substances may result from several routes of exposure (eg, oral and inhalation).

Sub-acute and chronic toxicity. Repeated small doses such as are found during medication or during industrial exposure may cause injury to quite different parts of the body from those affected by acute exposure. Animal tests attempt to reproduce this situation, the animals are exposed for 7, 30, 90 day periods or for 2 years to lifetime studies in the rat. The animals are observed for changes in behaviour, changes in the gross and microscopic structure, and changes in biochemistry of blood and tissues.

Carcinogenic effects. A carcinogen is a substance which causes cancer. Short-term carcinogenicity tests depend on the observation that a major group of carcinogens cause alterations to the chemical structure of the genetic material DNA. These genotoxic materials can be detected by their effects in causing mutation in bacteria or cells grown in the test tube (in vitro tests, eg, the Ames test). A number of chemicals cause cancer by hormonal or other mechanisms and do not show up in in vitro tests, thus whole animal studies are used. In such studies large numbers of animals have to be used because of the natural background of disease and cancer in all species. Mutations in human germ cells (sperm and ova) would also be harmful apart from carcinogenic effects and tests for mutagenicity are also carried out with this in mind.

Reproductive toxicity. Chemicals may interfere with reproduction at any stage from the production of sperm and ova to development of the foetus and newborn. Animals are dosed before mating, and during or after pregnancy. The development and numbers of offspring are assessed.

Mutagenicity. Chemicals may also cause permanent changes in genes which are passed along to descendent cells. A number of animal and in vitro tests cover the range of mutagenicity tests.

Irritancy/corrosivity. Substances can cause damage at the site of contact (skin, eye, etc) and tests for local irritancy and corrosive damage are carried out, particularly for industrial chemicals. (Irritation is the production of reversible tissue damage; corrosion is the production of irreversible tissue damage). Severely irritant properties of substances can usually be detected in vitro. Animal tests of eye irritancy (Draize test) can decide whether a substance is mildly irritant or non-irritant.

Table 2
EEC Directives which include Animal Testing Requirements

Directives by Subject Area	Status of Requirement
o **Trade in animals**	
Council Directive of 26th June 1964 on health problems affecting intra-community trade in bovine animals and swine (Directive 64/432/EEC) (OJEC, 29th July 1964), as amended by	Explicit in text
- Directive 80/219/EEC (OJEC L47, 21st February 1980)	Explicit in text
o **Animal nutrition products**	
Council Directive of 18th April 1983 on the fixing of guidelines for the assessment of certain products used in animal nutrition (Directive 83/228/EEC) (OJEC L126, 13th May 1983)	Explicit in text
Council Directive of 16th February 1987 fixing guidelines for the assessment of additives in animal nutrition (Directive 87/153/EEC) (OJEC L64, 7th March 1987)	Explicit in text
o **Veterinary Medicinal Products**	
Council Directive of 28th September 1981 on the approximation of the laws of the Member States relating to veterinary medicinal products (Directive 81/851/EEC) (OJEC, L317, 6th November 1981)	Implicit in safety cautions
Council Directive of 28th September 1981 on the approximation of the laws of the Member States relating to analytical, pharmaco-toxicological and clinical standards and protocols in respect of the testing of veterinary medicinal products (Directive 81/852/EEC) (OJEC L317, 6.11.81) as amended by	Explicit in text
- Directive 87/20/EEC (OJEC L15, 17th January 1987)	Explicit in text
o **Proprietary medicinal products**	
Council Directive of 26th January 1965 on the approximation of provisions laid down by law, regulation or administrative action relating to proprietary medicinal products (Directive 65/65/EEC) (OJEC, 9th February 1965), as amended by	Implicit

Table 2 (Continued)
EEC Directives which include Animal Testing Requirements

Directives by Subject Area	Status of Requirement
- Directive 83/570/EEC (OJEC L332, 28th November 1983)	Explicit in text
- Directive 87/21/EEC (OJEC L15, 17th January 1987)	Implicit in safety cautions
Council Directive of 20th May 1975 on the approximation of the laws of Member States relating to analytical, pharmaco-toxicological and clinical standards and protocols in respect of the testing of proprietary medicinal products (Directive 75/318/EEC) (OJEC L147, 9th June 1975), as amended by	Explicit in text
- Directive 83/570/EEC (OJEC L332, 28th November 1983);	Explicit in text
- Directive 87/19/EEC (OJEC L15, 17th January 1987)	Explicit in text
Council Recommendation of 26th October 1983 concerning tests relating to the placing on the market of proprietary medicinal products (Directive 83/571/EEC), (OJEC L332, 28th November 1983)	Explicit in text
Council Recommendation of 9th February 1987 concerning tests relating to the placing on the market of proprietary medicinal products (Directive 87/176/EEC) (OJEC L73 16th March 1987)	Explicit in text
o <u>Classification, Packing and Labelling of Dangerous Preparations</u>	
Council Directive of 26th June 1978 on the approximation of the laws of the Member States relating to the classification, packaging and labelling of dangerous preparations (pesticides) (Directive 78/631/EEC) (OJEC L206, 29th July 1978)	Explicit in text
o <u>Classification, Packing and Labelling of Dangerous Substances</u>	
Council Directive of 27th June 1967 on the approximation of laws, regulations and administrative provision relating to the classification, packaging and labelling of dangerous substances (Directive 67/548/EEC) (OJEC 196, 16th August 1967), as amended by	Implicit in classification and labelling requirements

Table 2 (Continued)
EEC Directives which include Animal Testing Requirements

Directives by Subject Area	Status of Requirement
- Directive 79/831/EEC (OJEC L259, 15th October 1979);	Explicit in text
o Food Additives	
Commission Recommendation of 11th November 1980 to the Member States concerning tests relating to the safety evaluation of food additives (Recommendation 80/1089/EEC) (OJEC L320, 27th November 1980)	Implicit Explicit in guidelines(1)
o Cosmetics	
Council Directive of 27th July 1976 on the approximation of the laws of the Member States relating to cosmetic products (Directive 76/768/EEC) (OJEC L262, 27th September 1976)	Implicit
Commission Decision of 19th December 1977 establishing a Scientific Committee on Costmetology (Decision 78/45/EEL) (OJEC L.13, 17th January 1978)	Implicit Explicit in guidance notes(2)

(1) Report of the Scientific Committee for Food on "Guidelines for the Safety Assessment of Food Additives (1980).

(2) Reports of the Scientific Committee on Cosmetology (Third Series) "Notes of Guidance for the Toxicity Testing of Cosmetic Ingredients" in EEC Environment and Quality of Life (1983).

Table 3
Types of Alternatives to Animal Testing

Mathematical Modelling of Structure-Activity Relationships and Computer Graphics, Network Thermodynamic Modelling
Mathematical modelling based on chemical structure: at an early stage of development.

Mathematical Modelling of Bio-chemical and Physiological Processes
Developed on the basis of experimental data, including animal testing.

Human Studies
Direct testing of substances intended for human use, eg, cosmetics (and toiletries), drugs.

Epidemiological research, particularly on substances to which humans are exposed indirectly, eg, pesticides, chemicals used in the workplace.

Use of Lower Organisms
Includes use of bacteria, algae, protozoa, coelenterates, fungi, plants; insects, echinoderms, molluscs.

In-Vitro Techniques
Cell cultures: obtained from human tissues or animals. In some cases a continuous cell line may be established from a primary cell culture; a great deal of work is being carried out in these areas.

Organ cultures: obtained from donor animals. Many organ cultures can be established from each donor animal, the cultures are comparatively short-lived.
Sub-cellular functions: e.g. enzymes, microsomes.

Improved Storage, Exchange and Use of Information
Making available to others data on experiments already carried out. (Possibly) permitting animal experimentation only where the results obtained are eventually made public.

Accepting results of toxicity testing carried out in other countries/by other authorities.

Harmonising regulations and test protocols across authorities.

Improved Design of Experiments
Design of experiments with statisticians' input may reduce the numbers of animals required, although there are arguments that better design can lead to increased numbers of animals.

Stepwise approach (see 2.2.3) can reduce extent of animal testing.

Sequential approach to acute toxicity testing reduces numbers of animals tested.

Source: Based on FRAME, Alternatives to Animal Experiments Frame, Nottingham, 1985.

Table 4
Summary of the Principal Test Areas where Alternatives are Being Developed

Test Areas	Level of Concern	Development of Alternatives
Acute Toxicity		
Appraisal of acute toxic potential required for chemicals which may be directly or indirectly (eg, through the environment) ingested or absorbed.	Classic measure is LD_{50}: widely criticised on animal welfare grounds and by some toxicologists.	Focus of attention in developing alternatives, particularly refinement approaches. Limit tests and approximate LD_{50} testing well-established in regulations though not always acceptable. Other refinement approaches being developed too.
Long-Term Toxicity		
Appraisal required for substances to which people may be directly or indirectly exposed over a long period.	Not under particular public scrutiny. Concern to develop alternatives includes users of the test because of cost and time involved.	May be scope for reducing the extent of testing in terms of period and dosage; potential role for in-vitro approaches for preliminary screening and identifying modes of action but more basic research needed to develop and assess realistic replacement approaches.
Carcinogenicity/Mutagenicity		
Evaluation required for substances to which people may be directly or indirectly exposed.	Focus of concern by public, users and regulators. Standard tests are difficult to evaluate and time-consuming and costly to run.	Area where replacement in-vitro approaches being developed and used in industry for screening but no alternatives yet acceptable for regulatory purposes.
Reproductive Toxicity		
Appraisal of inter-generation effects.	Test requirements have increased since thalidomide disaster. Area where pressure is strong for more testing.	In-vitro tests used in some R and D work, but not to great extent for regulatory purposes. Possible scope for reducing animal testing through harmonising test requirements and exchange of data.

Table 4 (Continued)
Summary of the Principal Test Areas where Alternatives are Being Developed

Test Areas	Level of Concern	Development of Alternatives
Dermal and Ocular Irritancy/Corrosivity		
Important for substances and products directly applied to skin or eyes or to which people may be exposed at the workplace.	Draize test in particular subject to much public/pressure group opposition.	Steps taken to refine procedures to reduce numbers of animals and limit pain and suffering. In-vitro alternatives and use of isolated eyes being developed towards regulatory acceptance.
Sensitivity Testing		
An expanding area as understanding of sensitivity/allergy grows.	Pressure for increased information on substances, but no standardised test approaches developed.	Area where there may be scope for research into non-animal, or other alternative approaches while test approaches are being developed.

Table 5
International Criteria for Classification of Chemicals based on LD_{50} Values

United Nations

Solid	Toxic 1 (<5)	Toxic 2 (<50)	Toxic 3 (<500)
Liquid	Toxic 1 (<5)	Toxic 2 (<50)	Toxic 3 (<2,000)

USA

Super Toxic (<5)	Highly Toxic (<50)	Very Toxic (<500)	Moderately Toxic (<5,000)	Slightly (<15,000)

Switzerland

Substance	Category 1 (<5)	Category 2 (<50)	Category 3 (<500)	Category 4 (<5,000)	Category 5 (<15,000)
Products	Category 1 (<5)	Category 2 (<50)	Category 3 (<500)	Category 4 (<2,000)	Category 5 (<15,000)

Japan

Toxic (<30)	Deleterious (<300)

European Economic Community

Very Toxic (<25)	Toxic (<200)	Harmful (<2,000)

United Kingdom/Pesticide Safety Precaution Scheme

Solid	Very Toxic (<5)	Toxic (<50)	Harmful (<500)
Liquid	Very Toxic (<25)	Toxic (<200)	Harmful (<2,000)

World Health Organisation

Solid	Extremely Hazardous (<5)	Highly Hazardous (<50)	Moderately Toxic (<500)	Slightly Toxic (<5,000)
Liquid	Extremely Hazardous (<20)	Highly Hazardous (<200)	Moderately Toxic (<2,000)	Slightly Toxic (<5,000)

NOTE:

The classification category (eg, Toxic 1) is followed by the LD_{50} values (in brackets) in mg/kg bodyweight which define the limit for a category.