ECETOC Document

No 30

Comments and Proposed Changes to OECD Screenings Information Data Set Draft Test Guidelines "Preliminary Reproduction Toxicity Screening Test" and "Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Test"

July 1992

T008/TROECD5

ECETOC DOCUMENT N° 30

COMMENTS AND PROPOSED CHANGES TO OECD SCREENING INFORMATION DATA SET DRAFT TEST GUIDELINES " PRELIMINARY REPRODUCTION TOXICITY SCREENING TEST" AND "COMBINED REPEAT DOSE AND REPRODUCTIVE/DEVELOPMENTAL TOXICITY SCREENING TEST"

Brussels, July 1992

TABLE OF CONTENTS

COMMENTS AND PROPOSED CHANGES TO OECD SCREENING INFORMATION DATA SET DRAFT TEST GUIDELINES; "PRELIMINARY REPRODUCTION TOXICITY SCREENING TEST" AND COMBINED REPEAT DOSE AND REPRODUCTIVE/DEVELOPMENTAL TOXICITY SCREENING TEST"

SUMMARY

In the OECD Existing Chemicals Programme a Screening Information Data Set (SIDS) has been defined. In this connection two new experimental guidelines have been written, a "Preliminary Reproduction Toxicity Screening Test" and a "Combined Repeat Dose and Reproductive/ Developmental Screening Test". The former has advantages in providing information relatively quickly information compared with conventional reproduction studies. The latter tries to evaluate target organ and reproduction toxicity within the same study and these two aims are not compatible in terms of doses required to investigate these two forms of toxicity. The guidelines have been reviewed and recommendations for change are made.

studies is required. This would require studies to be made according to the current OECD test guidelines 407 (for a 14 or 28-day study), 414 (for teratogenicity), and either 415 (one generation reproduction toxicity study) or 416 (two generation reproduction toxicity study). To carry out such a testing programme would be both resource and time consuming. Nevertheless the results gathered would allow a risk evaluation for man to be made.

In taking into account the large number of chemicals that need to be tested and the consequent resource use, OECD has considered mechanisms by which the toxic potential of a test substance may be evaluated by using a test programme employing a limited number of both animals and parameters to be studied.

To this end OECD have proposed two new test guidelines. The first, a "Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Test" assumes that no significant toxicological information is available and is essentially the combination of a single generation reproduction toxicity study with additional elements of histopathology, clinical chemistry and haematology normally associated with a subacute study. The second, a "Preliminary Reproduction Toxicity Screening Test" assumes that data from repeated dose toxicity studies is available.

It was anticipated by those compiling the two new draft guidelines that the resources required for conducting toxicological studies of this nature will be considerably less than conventional. Thus the new draft guidelines as defined are limited in their scope compared to conventional studies.

The intention of SIDS is to select between those products which should have the highest priority for immediate, further, full scale testing using conventional guidelines, and those to which a lower priority should be assigned. In the long term it is assumed that a minimum data set which will be generated for all chemicals will encompass repeat dose toxicity studies and developmental reproduction toxicity studies.

The two draft toxicity testing guidelines have been circulated to OECD Member States, but as yet they have not been accepted as official OECD test guidelines. It is reported that a number of laboratories are using these

2. EVALUATION OF THE DRAFT OECD-SIDS GUIDELINES

2.1. Commentary on the SIDS Guidelines

The Guidelines reproduced in appendices 1 and 2 were developed by a group of experts in a meeting held in January 1990 and subsequently revised in March, April and October 1990. The two protocols have been evaluated separately and our comments are placed alongside the original OECD text. These comments are further expanded in the following Section and are presented using the paragraph headings from the respective guidelines. Those paragraphs which are not mentioned do not require further discussion. For ease of expression the "Preliminary Reproduction Toxicity Screening Test" is subsequently referred to as the "Reproduction Test" and the "Combined Repeat Dose and Reproductive/ Developmental Toxicity Screening Test" as the "Combined Test".

1. Title and Foreword

The formulation of the Titles for both guidelines are not consistent. As both studies are **preliminary** and involve **reproduction and developmental** toxicity both key words should appear in the title. The term screening is confusing both in respect to its use in pharmacology and conventional toxicology and should be deleted.

As the tests to be performed according to these guidelines will not be sufficient for human risk evaluation there may be conflicts with National Animal Protection laws.

From a reader's standpoint it would be better to combine paragraphs 1 and 2 in both guidelines.

2. Introduction

Paragraph 4. Combined Test

The statement that "this test uses fewer animals of each sex per group" compared to a 28-day repeat dose study is wrong. In such a study 5

Paragraph 8 (Reproduction Test) and paragraph 9 (Combined Test)

Since gestation duration varies with rat strain it should be given as a range rather than as a fixed number of days. Consequently the maximum study duration also varies.

5. **Description of the Test Procedure**
Paragraph 10 (Combined Test)

Data concerning target organ toxicity must be interpreted with caution due to the use of rats for reproduction toxicity studies (aged 10-12 weeks) which are older than rats (aged 6-8 weeks) used in 28-day repeat dose studies. In addition the exposure times are prolonged and the so called "high-dose level" may be to low to produce target organ toxicity due to the need to avoid compromising reproduction toxicity.

6. **Test Conditions**
Paragraph 14 (Combined Test)

The aim of a conventional 28-day toxicity study is to demonstrate target organ toxicity by using quite high doses and accepting mortality in a limited number of animals. Conversely, the dose levels used in a reproduction toxicity study should not induce mortalities in order not to obscure reproduction/developmental findings. For proper evaluation of reproduction/developmental findings in the combined study it would be preferable to refer to **induction of slight signs of toxicity without compromising the general health of the test animals** as the criterion for high-dose selection. Even then the dose levels for the "Combined Test" may either be too high for the reproduction aspects or too low to demonstrate target organ toxicity adequately. In our opinion the two goals are irreconcilable and for this reason the guideline is seriously defect.

Additionally, in order to select acceptable dose levels, a dose-range finding study may be necessary; this contradicts the preliminary

(reproduction test). Killing the pups at day 4 will mask changes that occur only later during pup development.

9. Clinical Examination
Paragraphs 26-28 (Combined Test)

Haematological examinations can be performed only in male animals. Since the female animals undergo major physiological changes during pregnancy and lactation, the data gathered are not comparable to those from animals of conventional 28-day studies. The same holds for clinical chemistry and urine analysis. Therefore it should be clearly stated in the guideline that **all** clinical examinations (not just haematology) are performed in male animals only. The lack of background haematological and clinical chemistry for females at this age is a serious deficiency.

10. Pathology
Paragraph 25 (Reproduction Test) and paragraph 30 (Combined Test)

It is not feasible to distinguish between the number of corpora lutea graviditatis on day 4 after parturition since corpora lutea periodica, formed during the ongoing oestrus cycle, prevent such distinction. Counting corpora lutea at the time of killing is therefore of doubtful value and should be omitted.

In our opinion necropsy of apparently normal pups should not be required in a preliminary study because no substantial additional information on pup morphology will be achieved.

Paragraph 27 (Reproduction Test)

The epididymides and all organs showing macroscopic changes should be included in the list of organs to be preserved.

when placing chemicals in order of work priority when little is known of their intrinsic reproductive toxicity. From a positive standpoint the study can be considered to be equivalent to a pilot study conducted before the onset of a single or a two generation reproduction toxicity study and therefore can be justified in terms of usage of experimental animals. The study is deficient in that reduced litter size, decreased survival to day 4 and reduced pup weights provide only indirect and limited information regarding potential embryotoxic/teratogenic effects.

When evaluating the "Combined Test" guideline, including the amendments and comments made above, it is our opinion that the guideline has become very complex by attempting to answer too many questions. The major defect within the guideline is the incompatibility between the doses required for a repeat dose study to achieve target organ toxicity and those required to evaluate the reproduction process. In most instances users of the guideline have to conduct a range-finding study in order to define the correct dose. A decision has to be made whether to give greater weight to the reproductive aspects or target organ toxicity. The data obtained from the "repeat dose" section of the "Combined Test" are not equivalent to those obtained from a conventional 28-day study conducted in accordance with OECD Guideline 407.

2.3. Comparison of Experimental Costs

Cost Estimates for a test conducted according to the "Combined Test" guideline and the "Reproduction Test" guideline were obtained from the report authors' laboratories and from independent toxicology contract laboratories. As the cost basis varies between laboratories the estimates were compared and expressed as a ratio relative to the work required in a 28-day subacute study using OECD Guideline 407. Thus the cost of the "Reproduction Test" is 1.5 times and the "Combined Test" is twice the cost of a 28-day study (Table 1). Additional consideration should be given to the fact that the study results gathered from both draft protocols are only preliminary and that additional testing according to conventional study protocols may be subsequently necessary.

References

- OECD, (1981a). Organisation for Economic Co-operation and Development, Guidelines for testing of chemicals, Section 4, Health Effects, N°407, Repeated Oral Dose Toxicity, Rodent, 28-day or 14-day study.

- OECD, (1981b). Organisation for Economic Co-operation and Development, Guidelines for testing of chemicals, Section 4, Health Effects, N°414, Teratogenicity.

- OECD, (1983). Organisation for Economic Co-operation and Development, Guidelines for testing of chemicals, Section 4, Health Effects, N°415, One-generation reproduction toxicity study.

- OECD, (1986). Existing Chemicals. Systematic Investigation Priority Setting and Chemical Reviews.

- OECD, (1990a). Draft Protocol for a Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Test.

- OECD, (1990b). Draft Protocol for a Preliminary Reproduction Toxicity Screening Test.

- Wickramaratne, G.A. (1987). The Chernoff-Kavlock Assay: Its validation and application to rats. Teratog. Carcinog. Mutagen. 7, 73-83.

Table 1: Cost Estimates for Conducting OECD Proposed Toxicity Screening Tests relative to a subacute study expressed as unity using OECD Guideline 407.

	number of laboratories estimating	Estimated Cost (relative to OECD 407 = 1)	SD	Range
'Combined Test'	8	1.95	0.17	1.8-2.3
'Reproduction Test'	3	1.5	0	-
OECD 415 One Generation	8	4.05	0.90	3.0-6.0
OECD 414 Teratology	8	1.95	0.52	1.4-3.0
Chernoff-Kavlock (modified)	2	0.25		0.2-0.3

OECD TEXT (continued 1)	ECETOC COMMENT
LIMITATIONS OF THE METHOD	
6 This test does not provide complete information on all aspects of toxicity or of development. In particular, it offers no means of detecting delayed manifestations of prenatal exposure or effects that may be induced during postnatal exposure. Due (amongst other reasons) to the small numbers of animals in the dose groups, the selectivity of the end points, and the short duration of the study, the method will not provide evidence for claims of no effects.	
PRINCIPLE OF THE METHOD	
7 The test substance is administered in graduated doses to several groups of males and females. Males should be dosed during two weeks before mating which is considered sufficiently long to enable detection of a number of effects on male fertility and spermiogenesis.	
8 Females of the parental generation should be dosed for two weeks (with the objective of covering at least two complete oestrous cycles) in order to elicit any adverse effects on oestrus by the test substance. The animals are then mated. The test substance is administered to both sexes throughout the mating period and pregnancy and up to day 4 of lactation when all animals, including offspring, are killed.	Males should be killed 14 days after mating in order to reduce variability induced.
9 Maximum duration of the study following acclimatisation is 52 days, (14 days premating, (up to) 14 days mating, 22 days gestation, 4 days lactation).	Duration of gestation depends upon the strain of the rat. It is defined as 20 days in the "Reproduction test" guideline. The texts should be made consistent.
DESCRIPTION OF THE TEST PROCEDURE	
Preparation	
10 Healthy young adult animals are randomised and assigned to the treatment groups. The animals are kept in cages preferably in the same room for at least five days to allow for acclimatisation. It is recommended that the test substance be administered orally. Other routes of administration are also acceptable. All animals should be dosed by the same method during the appropriate experimental period. If any vehicle or other additive is used to facilitate dosing, it should be known not to produce toxic effects. Dosing should be on a seven-day per week basis.	The test has to use young adults in order to achieve reproduction; the lack of comparability with a conventional 28 day repeat dose study needs further emphasis.
EXPERIMENTAL ANIMALS	
Selection of Species	
11 This test guideline is designed for use with the rat. If other species are used, appropriate modifications will be necessary. Strains with low fecundity should not be used. Healthy virgin animals, not subjected to previous experimental procedures, should be used. The test animals should be characterised as to species, strain, sex, weight and/or age.	The wording used in this paragraph is marginally different to that used in the "Reproduction Test" Guideline. The texts should be made consistent.

OECD TEXT (continued 3)

ECETOC COMMENT

PERFORMANCE OF THE TEST

Experimental Schedules

16 Dosing of both sexes should begin 2 weeks prior to mating, after they have been acclimatised for at least five days. Mating should begin as soon as the animals have attained full sexual maturity. This may vary slightly for different rats in different laboratories, e.g. Sprague Dawley rats 10 weeks of age, Wistar rats about 12 weeks of age. Dosing is continued in both sexes during the mating period. Dams with offspring should be killed on day 4 post partum and other females on day 26 after their last mating. Males should be killed during the period when females are killed unless the protocol is modified to accommodate a second mating.

Problems are foreseen at the time at which males should be killed. Since cohabitation may last from 1 - 14 days (see Guideline paragraphs 9 + 20, treatment time for the males may vary from 24 - 53 days which could markedly affect blood chemistry. It is therefore recommended that all males be killed at a fixed time, eg at 28 days since it is unlikely that a protocol would be amended mid-experiment to accommodate a second mating.

17 Daily dosing of the parental females should continue throughout pregnancy and up to day 4 of lactation.

18 Consideration should be given to modifications in the dosing schedule based on available information on the test substance, such as induction of its metabolism or bioaccumulation.

Mating Procedure

19 1:1 (one male to one female) matings should be used in this study.

20 The female should be placed with the same male until pregnancy occurs or two weeks have elapsed. Each morning the females should be examined for the presence of sperm. Day 0 of pregnancy is defined as the day sperm is found.

What should happen when the male dies prior to mating is not clear; it is recommeded that a proven male from the same dosage group be used.

Litter Size

21 Animals dosed during the study are allowed to litter normally and rear their progeny to day 4 of lactation.

Observations

22 Throughout the test period, each animal should be observed at least once daily and more frequently when signs of toxicity are observed. Pertinent behavioural changes, signs of difficult or prolonged parturition and all signs of toxicity, including mortality, should be recorded. Signs of toxicity, when observed should include time of onset, degree and duration. Cageside observations should include changes in skin, fur, eyes and mucous membranes as well as respiratory, circulatory, autonomic and central nervous system functions.

OECD TEXT (continued 5)	ECETOC COMMENT
PATHOLOGY	
Gross Necropsy	
30 At the time of sacrifice or death during the study the animals should be examined macroscopically for any structural abnormalities or pathological changes. For parents special attention should be paid to the organs of the reproductive system. Further examination of infertile males or nonpregnant females is encouraged. The number of corpora lutea and implantation sites should be recorded. Dead pups and pups killed at day 4 should also examined macroscopically.	It is impossible to count corpora lutea at day 4 post partum as the next ovarian cycle has already commenced. This requirement should be deleted from the Guideline. Necropsy of apparently normal pups rarely divulges an abnormality, we recommend that autopsies could, without loss of significant information, be limited to pups which die.
31 The liver, kidney, thymus of all animals, and the testes and epididymides of all male parental animals should be weighed.	The adrenals should be added to the list.
Histopathology	
32 Liver, kidney, adrenals brain, heart, spleen, ovaries, seminal vesicles, testes and macroscopically abnormal tissues of all parental animals should be preserved for microscopic examination. Formalin fixation is not acceptable for examination of testes and epididymides. An acceptable method is the use of Bouin's fixative for these tissues embedded in paraffin.	The epididymides are missing from the preliminary list of tissues. Histology is increased compared to Guideline 407.
33 Histological examination should be performed on the preserved organs of the highest dose group and the control group. Examinations should be extended to the animals of other dosage groups when changes are seen in the highest dose group.	
DATA AND REPORTING	
Treatment of Results	
34 Individual animal data should be provided. Additionnally data may be sumarised in tabular form, showing for each test group the number of animals at the start of the test, the number of animals with lesions (including type of lesion and the percentage showing each type), the number of fertile males, the number of pregnant females and the types of changes.	
35 When possible, numerical results should be evaluated by an appropriate and generally accepted statistical method.	

OECD TEXT (continued 1)	ECETOC COMMENT
7 Females of the parental generation should be dosed for two weeks (with the objective of covering at least two complete oestrous cycles) in order to elicit any adverse effects on oestrus by the test substance. The animals are then mated. The test substance is administered to both sexes throughout the mating period and pregnancy and up to day 4 of lactation when all animals, including offspring, are killed.	Males should be killed 14 days after mating.
8 Duration of the study, following acclimatisation, is 52 days (14 days premating, (up to) 14 days mating, 20 days gestation, 4 days lactation).	Duration of gestation varies with the strain of the rat. It is defined as 22 days in the "Combined Test" guideline.
DESCRIPTION OF THE TEST PROCEDURE	
Preparation	
9 Healthy young adult animals are randomised and assigned to the treatment groups. The animals are kept in cages for at least 5 days to allow for acclimatisation. It is recommended that the test substance be administered orally. All animals should be dosed by the same method during the appropriate experimental period. If any vehicle or other additive is used to facilitate dosing, it should be known not to produce toxic effects. Dosing should be on a seven-day per week basis.	
EXPERIMENTAL ANIMALS	
Selection of Species	
10 This test guideline is designed for use with the rat. If other species are used, appropriate modifications will be necessary. Strains with low fecundity should not be used. Healthy animals, not subjected to previous experimental procedures, should be used. Females should be nulliparous and non-pregnant. The test animals should be characterised as to species, strain, sex, weight and/or age.	The wording used in this paragraph is marginally different to that used in the "Combined Test" Guideline
Number and Sex	
11 Each test and control group should contain a sufficient number of animals to yield about 8 pregnant females at near term in the absence of an adverse reproductive effect. The objective is to produce enough pregnancies and offspring to assure a meaningful evaluation of the potential of the potential of the substance to affect fertility, pregnancy and maternal behaviour and suckling, growth and development of the F1 offspring from conception to day 4 of lactation.	The comparison between numbers of animals used in this protocol and those employed in a single generation reproduction study should be reinforced.
Housing and Feeding Conditions	
12 The temperature in the experimental animal room should be 22°C (+/-3°) and the relative humidity 30 to 70 per cent. When the lighting is artificial the sequence should be 12 hours light, 12 hours dark. For feeding conventional laboratory diets may be used with an unlimited supply of drinking water. Pregnant females should be caged individually and may be provided with nesting materials.	

OECD TEXT (continued 3)	ECETOC COMMENT
Observations	
21 Throughout the test period, each animal should be observed at least once daily. Pertinent behaviour changes, signs of difficult or prolonged parturition and all signs of toxicity, including mortality should be recorded.	It is not possible to make specific behavioural tests in pups, but obvious behavioural abnormalities should be reported. Para. 22 of the "Combined Test" is more explicit.
22 During pre-mating and mating period, food consumption should be measured at least weekly. Optionally, food consumption may be measured during pregnancy. After parturition, and during lactation, food consumption measurements should be made on the same day that litters are weighed. Males and females should be weighed on the first day of dosing, at least weekly thereafter and on the day of termination. These observations should be reported individually for each adult animal.	Food consumption is of doubtful value during cohabitation. Paragraph 23 of the "Combined Test" is more explicit.
23 The duration of gestation should be calculated from day 0 of pregnancy. Each litter should be examined as soon as possible after delivery to establish the number and sex of pups, stillbirths, live births and the presence of gross anomalies.	
24 Live pups should be counted and sexed and litters weighed on the morning after birth and on day 4. Physical and/or behavioural abnormalities observed in the dams or offspring should be recorded.	It is not possible to make specific behavioural tests in pups, but obvious behavioural abnormalities should be reported. Behavioural abnormalities in dams should be deleted as these effects are covered in para 21.
PATHOLOGY	
Gross Necropsy	
25 At the time of sacrifice or death during the study the animals should be examined macroscopically for any structural abnormalities or pathological changes, with special attention paid to the organs of the reproductive system. Further examination of infertile males or nonpregnant females is encouraged. The number of implantation sites and corpora lutea should be recorded. Dead pups and pups killed at day 4 should also examined macroscopically.	It is impossible to count corpora lutea at day 4 post partum as the next ovarian cycle has already commenced. This requirement should be deleted from the Guideline. Necropsy of apparently normal pups rarely divulge an abnormality, we recommend that autopsies could without loss of significant information, be limited to pups which die.
26 The testes and epididymides of all male parental animals should be weighed.	
Histopathology	
27 The ovaries and testes of all parental animals should be preserved for microscopic examination. Formalin fixation is not acceptable for the examination of the testes and epididymides. An acceptable method is the use of Bouin's fixative for these tissues embedded in paraffin.	Epididymides should be added to the list of tissues for histological examination together with any tissue showing macroscopic lesions. Suggest the first sentence is changed to: The ovaries, testes and epididymides as well as all organs showing macroscopic lesions of all parental animals should be preserved for microscopic examination.
28 Histological examination should be performed on the preserved organs of the highest dose group and the control group. Examinations should be extended to the animals of other dosage groups when changes are seen in the highest dose group.	For clarification purposes it would be better to say "preserved reproductive organs" in the first sentence.

Appendix 3 (cont.1). Comparison of Guidelines 407, 414 AND 415 with OECD-SIDS "Combined Test" and "Reproduction Test".

	OECD (1981a) TG 407 28-day subacute study	OECD (1981b) TG 414 Teratology Study	OECD (1983) TG 415 Single generation reproduction	OECD-SIDS (Draft) (1990a) "Combined Test"	OECD-SIDS (Draft) (1990b) "Reproduction Test"
TREATMENT					
5. Dose levels	at least 3 + control	at least 3 + control + also vehicle control if the test substance is administered in a vehicle	at least 3 + control or vehicle control receiving the vehicle in the highest volume used + paired-fed control if the test substance cause reduced dietary intake	at least 3 + control or vehicle control if the test substance is administered in a vehicle	at least 3 + control or vehicle control if the test substance is administered in a vehicle
- low dose	no evidence of toxicity (but higher than estimated human exposure)	no observable effects	ideally, no observable adverse effects on the parents or offspring	to provide meaningful evaluation of dose-response	to provide meaningful evaluation of dose-response
- intermediate dose(s)	minimal toxic effects	geometrically between high and low dose	ideally minimal toxic effects	to provide meaningful evaluation of dose-response	to provide meaningful evaluation of dose-response
- high dose	toxic effects but not an incidence of fatilities which would prevent a meaningful evaluation	ideally, some overt maternal toxicity (e.g. slight weight loss, but not more than 10% maternal deaths) unless limited by the physical/ chemical nature or biological properties of the substance	ideally, toxicity but no mortality in the parental animals, unless dose is limited by physical/chemical nature or biological effects	toxicity but no mortality in the parental animals	toxicity but no mortality in the parental animals
limit test	1,000 mg/kg bw/d	1,000 mg/kg bw/d	1,000 mg/kg bw/d	1,000 mg/kg bw/d	1,000 mg/kg bw/d

Appendix 3 (cont.3). Comparison of Guidelines 407, 414 AND 415 with OECD-SIDS "Combined Test" and "Reproduction Test".

	OECD (1981a) TG 407 28-day subacute study	OECD (1981b) TG 414 Teratology Study	OECD (1983) TG 415 Single generation reproduction	OECD-SIDS (Draft) (1990a) "Combined Test"	OECD-SIDS (Draft) (1990b) "Reproduction Test"
PROCEDURE					
9. Mating		with M of established fertility or by artificial insemination	1:1 (1 F is placed with the same M until pregnancy occurs or 3 wk have elapsed)	1:1 (1 F is placed with the same M until pregancy occurs or 2 wk have elapsed)	1:1 (1 F is placed with the same M until pregancy occurs or 2 wk have elapsed)
			alternatively, 1 M : 2 F		
10. Proof of fertility			pairs that fail to mate should be evaluated to determine the cause of infertility	not specified	not specified
11. Rearing: Litter size					
- without standardisation			dams are allowed to litter normally and rear their progeny to the stage of weaning	dams are allowed to litter normally and rear their progeny to day 4 of lactation	dams are allowed to litter normally and rear their progeny to day 4 of lactation
- with standardisation			on d 4 after birth, selection of 4 M and 4 F per litter (as nearly as possible)		

Appendix 3 (cont.5). Comparison of Guidelines 407, 414 AND 415 with OECD-SIDS "Combined Test" and "Reproduction Test".

	OECD (1981a) TG 407 28-day subacute study	OECD (1981b) TG 414 Teratology Study	OECD (1983) TG 415 Single generation reproduction	OECD-SIDS (Draft) (1990a) "Combined Test"	OECD-SIDS (Draft) (1990b) "Reproduction Test"
16. Mortality	observe at least twice daily	observe at least twice daily	not specified	not specified	not specified
17. Examination of litters at birth			as soon as possible after delivery	as soon as possible after delivery	as soon as possible after delivery
			number of pups, still-births, live births	number of pups, still-births live births	number of pups, still-births, live births
			sex of pups	sex of pups	sex of pups
			gross anomalies	gross anomalies	gross anomalies
18. Preservation of pups			dead or moribund pups and pups sacrificed at d 4 should be studied for possible defects	dead pups and pups killed at d 4 should be examined macroscopically	dead pups and pups killed at d 4 should be examined macroscopically
19. Examining during lactation			counting of live pups; weighing of litters of the morning after birth, and at d 4, d 7 and weekly thereafter until termination of the study when animals should be weighed individually	counting of live pups; sexing and weighing of litters on the morning after birth and on d 4	counting of live pups; sexing and weighing of litters on the morning after birth and on d 4
			physical or behavioural abnormalities in dams and offspring	physical or behavioural abnormalities in dams and offspring	physical or behavioural abnormalities in dams and offspring

Appendix 3 (cont.7). Comparison of Guidelines 407, 414 AND 415 with OECD-SIDS "Combined Test" and "Reproduction Test".

	OECD (1981a) TG 407 28-day subacute study	OECD (1981b) TG 414 Teratology Study	OECD (1983) TG 415 Single generation reproduction	OECD-SIDS (Draft) (1990a) "Combined Test"	OECD-SIDS (Draft) (1990b) "Reproduction Test"
26. Histopathology	all animals of the high dose and control groups; gross lesions; may be extended to animals of the low and intermediate dose group	not mentioned	if organs have not been examined in other multiple dose studies; all animals in control and high dose group	all animals in control and high dose group; examinations should be extended to animals of other dosage groups when changes were seen in the highest dose group	all animals in control and high dose group; examinations should be extended to animals of other dosage groups when changes were seen in the highest dose group
			all animals dying during study (where practicable). Organs showing abnormalities in these animals should be examined in all other parent animals		
			microscopy of all tissues showing gross pathological changes		
			microscopy of reproductive organs of animals suspected of infertility	ovaries optional but on any females failing to carry their pregnancy to term	ovaries optional but on any females failing to carry their pregnancy to term

APPENDIX 5

MEMBERS OF THE ECETOC SCIENTIFIC COMMITTEE

(Peer Review Committee)

* Stewards - responsible for primary peer review

W.F. TORDOIR (Chairman), Head of Occupational Health and Toxicology Division	SHELL NL - Den Haag
H. VERSCHUUREN, (Vice-Chairman) Head of Toxicology Department	DOW CHEMICAL CH - Horgen
O.C. BOECKMAN*, Scientific Advisor	NORSK HYDRO N - Porsgrunn
N.G. CARMICHAEL, Toxicology Director Worldwide	RHONE-POULENC F - Lyon
H. DE HENAU, European Technical Centre Professional and Regulatory Services	PROCTER AND GAMBLE B - Brussels
A. DE MORSIER, Head, Ecotoxicology	CIBA-GEIGY CH - Basel
P.A. GILBERT, Head, Environmental Relations	UNILEVER UK - Port Sunlight
I.J. GRAHAM-BRYCE, Head of Environmental Affairs	SHELL NL - Den Haag
B. HILDEBRAND*, Director, Experimental Toxicology	BASF AG D - Ludwigshafen
J.R. JACKSON, Director Medicine and Health Science	MONSANTO EUROPE B - Brussels
K. KUENSTLER, Head of Toxicology Department	HENKEL D - Duesseldorf
H. LAGAST, Chief Medical Officer	SOLVAY B - Brussels
E. LOESER, Head of Institute of Industrial Toxicology	BAYER D - Wuppertal
R. MILLISCHER*, Chief Toxicologist	ATOCHEM F - Paris
I.F.H PURCHASE, Director, Central Toxicology Laboratory	ICI UK - Macclesfield