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Proposed Health Effects Test Standards for Toxic Substances Control ACT Test Rules

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BEFORE THE ENVIRONMENTAL PROTECTION AGENCY

COMMENTS OF THE

EUROPEAN CHEMICAL INDUSTRY ECOLOGY AND TOXICOLOGY CENTRE

(ECETOC)

ON

PROPOSED HEALTH EFFECTS TEST STANDARDS FOR TOSCA

TOXIC SUBSTANCES CONTROL ACT TEST RULES

PROPOSED HEALTH EFFECTS TEST STANDARDS FOR TOXIC SUBSTANCES CONTROL ACT TEST RULES, 44 FED REG. 27334 (MAY 9, 1979) AND 44 FED. REG. 44059 (JULY 26, 1979)

Dockets N° Ots-046003 and 046005

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GENERAL ECETOC COMMENTS ON EPA HEALTH EFFECTS STANDARDS

INTRODUCTION :

A. The aims of the Standards

The EPA on page 27334, column 1 states that the ultimate purpose of the standards under consideration should be "to assure that data... can be used... to determine whether the tested chemicals present an unreasonable risk of oncogenic or other chronic effects, and to support regulatory actions to eliminate or reduce such risk." ECETOC shares the view that such data must be of adequate quality in all respects, but does not believe that the EPA proposals will lead to the achievement of such quality. Indeed, there are important aspects of these proposals which will have the contrary effect, and will lead to a worsening in the protection of health from the possible adverse effects of chemicals. This proposition is developed in the following sections.

B. Impact of EPA Standards on the Effectiveness of Toxicology

In order that it may yield good data, toxicological testing has to be carried out with a considerable degree of freedom to assess what is happening as the tests proceed, and to react to this assessment in the light of available experience and expertise. Only in this way can the maximum of useful and valid information be derived from a test.

The EPA Standards lay down in great detail the way in which every operation in toxicity testing shall be carried out. This attempt to codify what should by no means be a routine operation is incompatible with good toxicology, and hence with the production of good information.

C. Effect of EPA Standards on Scientists

In that part of the Standards dealing with the qualification of personnel employed in testing, the EPA rightly insists that "Perhaps the most critical element in the design and conduct of chronic effects studies is that of having knowledgeable and motivated scientific staff managing and conducting the studies".

ECETOC submits that possibly the major harmful effect of the EPA Standards, if adopted in their present form, would be to de-motivate staff. There is no point in requiring highly-qualified scientists while at the same time reducing their ability to act as highly-qualified scientists. Such people will under those circumstances prefer not to work on testing for legislatory purposes. This is a particularly counter-productive aspect of the EPA proposals, since it will actually lead to a lowering of the overall standard and motivation of scientists doing such work.

D. Testing Guidelines

The adoption of rigid and over-detailed test standards would constitute a very serious hindrance to discovery. Furthermore, to enshrine approved current techniques in legislation would delay the introduction of valuable new knowledge and techniques into such testing . At best, the time of valuable experts would be wasted in following due procedure for getting their discoveries adopted. All of this would be ultimately detrimental to health protection. In view of the above comments ECETOC strongly recommends EPA not to issue test standards in the form of regulation but to produce them as guidelines which are not obligatory and which avoid the dangers discussed above.

This would permit the full deployment of scientific expertise, would not delay the introduction of valuable new techniques, and at the same time would give the notifier a valuable insight into the sort of testing required by EPA.

In addition to these comments on principles, ECETOC has carefully reviewed the proposed standards in detail and makes the following points of criticism , with constructive suggestions for improving some of the points criticised.

The fact that the comments are so numerous serves to show how difficult it is to write toxicological tests into law, and reinforces our contention that the EPA should issue testi guidelines, not legislation.

I. COMMENTS APPLYING TO BOTH DOCUMENTS

1. Scope

The proposals apply to testing under section 4 of TSCA. The requirements for study-plan submission, interim reporting, and the determination of stability before testing would be inappropriate if carried over into section 5 testing of new chemicals, and we urge that this be avoided. Even for testing under Section 4 of TSCA, these same requirements should not be applied where studies have already been carried out before a section 4 requirement to test is made known. Data from such work should be admissible.

2. Reporting requirements

A number of comments on various aspects of reporting are collected here.

2.1. The submission of Interim Quarterly Summary Reports.

This is not justified for work of this nature, and is over-burdensome on qualified staff. Account should be taken of existing obligations to report confirmed, interim results where testing of a currently-manufactured chemical shows severe toxic or oncogenic effects.

2.2. Study plan.

The submittal of a Study Plan to EPA, prior to a study, should not be obligatory. If it were, then studies performed for purposes other than submission to EPA, e.g. for other authorities, would not be submittable. They should not be excluded. If a Study Plan is submitted, EPA should comment on it and give justification for its comments, especially if these are concerned with proposed deviations from the TSCA standards.

The Study Plan itself should contain the minimum details which are in compliance with the FDA requirements in Fed. Reg. 44, 247, Dec. 22, 1978, paragraph 58.120. The EPA proposals for the content of the Study Plan (p. 27351, f.2) are excessive and impracticable.

2.3. Data required in test report (p. 27352, 772.113, (k), (2))

The final test report requirements are over-elaborate, and inclusion of some of the detailed data is not necessary. This comment applies generally to the test standards. Only data relevant to the desired evaluation should be included in the final report, and the rest stored as raw data. For example, the sections on time of killing (2.i. A.4), time of necropsy (2.i A.5.) and the description of toxic signs, etc... (2.i B.3.) should be omitted, as they are stored as raw data anyway.

2.4. Reporting format (p. 27339, col 1)

The provision by EPA of an overall structure for the reporting of data would be helpful to notifiers. However, the proposition to have "specific standards for data formatting" is excessively elaborate and unreasonable. Test protocols, and hence the form of the data, may vary from case to case. Many laboratories world-wide have their own computer-based systems, the print-outs from which should be acceptable to EPA. Thus, on p. 27352 (2) "Data reporting" this should read : "Data reporting. The test report must contain the following information, arranged and presented in a clear, readable and checkable form".

2.5. EPA should make it clear that reporting to EPA is to be by the sponsor, not by the tester if he should be different from the sponsor. On page 27351, 772.113-1, (j.), the tester is required to submit certain data to EPA. This should read : "the sponsor must submit..."

3. Transitional period

There is no allowance for a transitional period, during which results from tests started before the introduction of the EPA proposals can be admissible. This should be allowed for.

II COMMENTS ON CHRONIC HEALTH EFFECTS STANDARDS

1. Benign neoplasms : p. 27350, 772.113-1,(c)2.

p. 27350, 772.113-1, (c) 2.

This sentence on the definition of "Oncogenic effects" should be followed by a sentence as follows : "When benign neoplasms are taken into account for evaluating carcinogenic potential, careful consideration must be given to associated chronic toxic effects."

2. Test or control substance concentration : p. 27351, 772.113-1, (g).

This rigid requirement for an actual concentration within ± 5% of the nominal concentration, and that variability of the actual concentration shall not exceed 10% between samples of the test mixture, is frequently unattainable in practice because of limitations in analytical procedures or in instrumentation.

The requirement for stability studies to be conducted before a study starts is also unreasonable. The stability of the substance itself, over, fo example, a 2-year period is often not available before a 2-year study commences, and the FDA requirement permitting concurrent stability testing (FR 43, 247, Dec. 22, 1978, para. 58.105, page 60017) is reasonable and practicable.

We suggest that this part should read as follows : " The sponsor must document the actual concentration of the test substance in the preparation administered to the animals for every concentration used. Before experiments are started with administration of the test substance in food or drinking water, properly conducted determination of the stability and homogeneity of the substance in the mixture should be m to permit the frequency of diet-preparation and monitoring to be established

3. Dietary requirements : p. 27351, 772.113-1, (h).

The specified diet is obtainable only in the USA, and it is not practicable for non-US laboratories to obtain supplies. Alternative well-defined diets which are nutritionally adequate and acceptable to the test animals should be permitted. Such diets exist and have been used extensively, so that considerable experience of their use has accumulated. Appendix A should be regarded as an example of an acceptable diet.

This section should therefore read :

(h) (1) : "For the chosen diet, the tester must ensure that the nutrient quality is adequate and that the level of contaminants is acceptable in the basal diet. Dietary constituents known to influence carcinogeniity must not be present in interfering concentrations. "

(h) (2) : " The tester must not use feed after its expiry date".

4. Analysis of feeds : p. 27351, 772.113-1 (i).
This should be omitted as it is covered by the requirement in (h) (1) above.

5. Reporting requirements : p.27351, col.3,(j). According to section I,2.1 and 2.5 this paragraph should read :"(1) If the substance under test is currently manufactured, the sponsor should inform EPA of any severe toxic or oncogenic effects arising during the course of the study. (2) Final test report."

6. Para (k) 1 iii) G on p. 27351 should read : "Age, body weight range, and condition ..... study".

7. Para. (k) 1. iv), delete : "amount of diet (ad libitum, consumption), amount of water", since these are already covered under viii) "methods of observation".

8. Para (viii)(E) on p.27352,col 1 should read :"Frequency of measuring consumption of feed in case of feeding study and water in case of administration via drinking water".

9. In para. (i) A (2), on page 27352, the"method of kill" should be omitted as it must be included under (k) (1), "description of test procedures". Thus, (i) A (2) should read : "Whether it was a scheduled kill, or whether the animal was found moribund or dead."

10. Para. (i) A (3) on page 27352 can be omitted because this is covered on page 27351, col. 3, iii) G.

11. Para (i) A (4) and (5) on page 27352, col. 2 should read : "Day of study on which death occured". The remaining dates are in raw data (see 2.3. in section I, General comments).

12. Para .(i)A (6) on page 27352 col.2, should be omitted since such information is difficult to define and irrelevant.

13. Para (i)B on page 27352, col.2, should be omitted because the listing of adverse clinical effects by test group (see No.17 below) is completely

14. Para. (i) C on page 27352, col. 2, should be entitled : "Food/water consumption and body weight data". Food consumption cannot be tabulated for each animal since they are normally caged in groups. Body weight and mean food consumption are usually determined simultaneously. Therefore C (2) should read : "Estimated food consumption (feeding study) and estimated water consumption (administration via drinking water), measured at weekly intervals for the first 13 weeks, and monthly for the remainder of the test period."
C (3) should read : "monthly" instead of "bi-weekly".

15. Para. (i) D (1) page 27352, col. 3. This is unnecessarily elaborate and should read : "For each animal, its identification number, the length of time on study, the moment of death and a complete description and diagnosis of every neoplasm and other lesions, together with clinical and gross observations. If a system for grading lesions is used, a description of the system must be submitted. The description and ..... must be noted". (3) can be deleted as it is covered in the above para.

16. Para. (ii) By test group, page 27352, col. 3. The words : "in the format specified" should be replaced by : "in a clear, readable and checkable form". See section I, general comments, 2.4.
The requirement for a "standard deviation" should be "as appropriate".

17. Para (A), page 27352, col. 3. This should be entitled : "Adverse clinical effects", and should read : "All toxic effects observed during the study should be reported for each test group (dose level and sex), including (1) : the number of animals affected; (2) the day of the study on which the effect occured and the median time from the start of the study to the first observation of such a response; and (3) the median period of dosing at death for those animals displaying each time of response".

18. Para. (B), page 27353, col. 1. Under (1) delete "estimated" and under (2) change "bi-weekly" to "monthly".

19. Para (C), page 27353, col. 1. The title should be "mortality data" and the requirement simplified to read :
"In an appropriate form, cumulative mortality data must be submitted for each test group".

20. Para D, page 27353,col 1. Section (1) should be moved to para (i) on "individual animals".

21. Para (E), page 27353, col 1. The groupwise listing of gross lesions gives no valuable information, and does not allow the original data reporting to be checked. Rather than improving the interpretation of the data, this could hinder it because of the usual discrepancies between the incidence of macroscopic and microscopic findings.

Para (E)(3) should be moved to the section on individual animals. Thus para.(E) should read:"Organ weights. Data showing the mean weight of each type of organ, the mean organ-to-body weight ratios and corresponding standard deviations arranged by test group (dose level and sex) must be supplied in tabular form."

22. Para (G)(2), page 27353, col 2. When a grading of lesions is used, all grades are to be listed separately. The averaging of grades is not defensible scientifically. Thus "the average grade (when applicable) of each type of lesion" should be deleted.

23. Para (H), page 27353, col. 2. H(2),(3) and(4) should be simplified to re "The cumulative incidence of tumors and other lesions should be submitte in an appropriate form if additional information is considered necessary".

24. Para (J), page 27353, col. 3. Historical controls are not always available, but data from them are of undoubted use. This para. should read :'Data on tumors and other lesions in historical controls may be used if available, to aid interpretation of the results'.

25. Para iii), page 27353, col. 3. In the first sentence the reference to gross necropsy findings and histopathology should be replaced by "and pathology".

26. Para (3), page 27353, col. 3. It is not scientifically correct to use a summary document for evaluating a toxicological study. Therefore the second sentence should read : "The summary must highlight any and all data or observations which may indicate toxic effects." The final sentence should be omitted.

27. Appendix A on page 27353 should be omitted. See our comment no.3 on diet above.

28. Para (a)(1)(i) and (ii), page 27354, col.3. The last sentence in each of these paras should be omitted. The same applies to the last sentence of para (3) on age at start of test.

29. Para (5), page 27355, col. 1. For "identical" substitute "similar".

30. Note (ii) on page 27355, col.1 should be deleted. To use known carcinogens as control in longterm studies presents an unreasonable risk to personnel, not justified by the data generated. Also, the exposure of laboratory personnel to known carcinogens is governed by various national laws in Europe and elsewhere.

31. Para (6), page 27355, col. 1. In Part 771 the route of administration should be judged on its merits by the appropriate qualified scientist. The last two sentences of (6) should therefore be deleted.

32. Para (7), page 27355, col 1. The second sentence should read. "For gavage the test substance must be administered for a minimum of 5 days per week for feeding ad libitum ; for inhalation exposure, a minimum of 5 days per week for 4 hours a day to the finally achieved actual concentration for any group on test."
Administration by gavage is extremely personnel -intensive and time - consuming, and the above conforms to current practice in Europe. In inhalation studies, the handling of animals, eg in transfer to inhalation devices, clinical observation, weighing etc., would not be compatible with normal working hours if the exposure were for 6 hours.

33. Para (8), page 25355, col. 1. This section needs more detail regarding various species, as follows :

(i) For rat studies the test substance must be administered for a minimum of 24 months ; the duration should be at least 30 months (6 months observation period), or alternatively the study should be terminated when the number of survivors in any of the lower dose groups or control group is reduced to 25% of the starting number for each sex individually.

(ii) For mouse studies the test substance must be administered for a minimum of 18 months ; the duration of the experiment should be at least 24 months (6 months observation period), or alternatively the study should be terminated when the number of survivors in any of the lower dose groups or control group is reduced to 25 % of the starting number for each sex considered separately.

(iii) For oncogenicity studies with other species, the tester must ensure that administration of the test compound lasts over a half of the expected median lifespan, and that the duration of the experiment including the observation period covers most of the life expectancy of the species used."

34. Para (9),(i), page 25355, col.1. The first sentence should be followed by :"Exceptions to these requirements are possible, eg for special inhalation studies with dusts or fibres . The exceptions shall be justified by the sponsor".

35. Para (ii), page 27355, col.2. The requirements for a range-finding study should be less rigid. We suggest the wording :
"The tester must conduct an appropriate range finding study to select the dose levels for an oncogenicity study.
Data other than from the preliminary study, derived for example from metabolic or pharmacokinetic studies, could be considered in selecting dose levels for the oncogenicity study".

The nature of the range-finding experiment depends on the nature of the compound, its accumulation, the animal species and the type of administration.

36. Para (C), page 27355, col. 2. In selecting the lowest dose level there are special cases where a wider range is needed than is represented by a factor of 10, eg in evaluation of dusts. Thus, this para. should read : "The lowest dose should not normally be less than 10 % of the highest dose. Intermediate dose levels should be a function, eg log increments, constant factors etc of these dose levels".

37. Para (iii), page 27355, col.2. The rationale for dose selection should be submitted in the test report, not in the "plan submission".

38. Para (i), page 27355, col.3. In an oncogenic study the only purpose of haematological testing is to detect leukaemia, and therefore interim haematology is not justified. It should be done on all animals at termination of the study. This implies also the deletion in para.(iii), of the phrase in brackets "other than..... this section".

    In para.(iv) the sentence "A technical employee... is killed" should read : "A technical employee should obtain blood samples from each animal killed during the study".

39. Para.2(i) (B), page 27356, col. 1. In the majority of cases a period between death and necropsy of about 24 hours does not prevent adequate histopathological examination provided the carcass is refrigerated as specified in the second sentence. Therefore the first sentence of this para. should read.: "Animals must be necropsied as soon as possible after death but no later than on the next day".

40. Para. (C), page 27356, col. 1. The second sentence should read : "The examination must include the following : external and internal portions of all hollow organs; cranial cavity and external surfaces of the brain; thoracic, abdominal, and pelvic cavities with their associate organs, and tissues; and the muscular/skeletal carcass."

    It is impossible and unnecessary to perform external examination of the spinal cord for all animals in routine experiments. Additionally, recent experience has shown that in the case of some chemicals, mediodorsal aspects of the nasal cavity in rodents are among the most sensitive parts of the mucosa. Opening of the nasal cavity for gross inspection will distort these parts of the nasal mucosa. Examination of the nasal cavity and paranasal sinuses should therefore be performed at the microscopic level only.

The last sentence of this para. should be omitted since the inflation of lungs and of the urinary bladder are methods which are debatable and often not necessary; any decision whether and how to carry it out should be left to the qualified pathologist.

41. Para. (ii), page 27356, col.1 should read :"Tissue preservation. A technical employee must immediately preserve the tissues and organs from all test animals regardless of their time of death in buffered formalin or another recognized and accepted fixative appropriate for the specific tissue (s) or the staining method foreseen. The following tissues must be preserved either as sections or in total, as is necessary with respect to appropriate fixation, for the appropriate time for the fixative utilized :"

42. Sections (A) to (P) of para. (ii), page 27356, col. 1 and col. 2. For a few organs or tissues there seems to be no justification for mentioning them as possible extras, either because neoplastic changes in these organs will easily be detected macroscopically (e.g. fallopian tube or some of the male accessory sex organs) or because a randomly-selected section of tissue will not be of any help to detect tumors (e.g. skeletal muscle). Therefore sections (C), (F), (G), (M), (O) and (P) of this para. should read :
(C) "Eyes",
(F) "Oral mucous membrane (e.g. from tongue, pharynx etc.)";
(G) "Heart";
(M) "Bone including marrow (sternum, vertebra, or tibio-femoral joint)";
(O) "for males : testes, prostate";
(P) "For females : vagina, corpus and cervix uteri, and ovaries."
Section (N) "Skeletal muscle;" should be deleted.

43. Para. (iii) (A), page 27356, col. 2 . Since fixation is part of the preservation described before under para.(ii), page 27356, col.1, this para. should be entitled "Trimming", and the first sentence should be deleted. The second sentence should read : "A pathologist must perform tissue trimming or be immediately available for supervision".

The third sentence "Routinely..." and the last sentence of this para. should be omitted because the qualified pathologist should be free to decide upon appropriate measures for tissue trimming and embedding from case to case.

44. Para. (iii) (B), page 27356, col. 2. The first two sentences should read : "A technical employee must cut all tissues foreseen for microscopic examination, as specified under (b) (2) (iv),(v) and (vi) of this section, at a thickness allowing for proper examination. All tissues must be stained with a suitable stain (e.g. hematoxylin-eosin)".

The fourth sentence of this para. should also be changed : "In case of focal gross lesions which are not evident histologically further slides should be prepared to confirm the necropsy findings".

45. Para. (iv) (A), page 27356, col.3. In the first sentence the words "evaluation with" must be deleted. The second sentence should read : "The same pathologist should examine and evaluate all microscopic slides, as defined in the next paragraph for one test in a given species".

46. Para. (iv) (B), page 27356, col.3. The first sentence should be changed and extended : "Microscopic examination should be performed at least on all tissues described in paragraphs (b) (2) (ii), and (b) (2) (vi) of this section in all animals which died or were killed in moribund conditions during the experiment as well as in all animals of the highest dose and control groups. In case of reduced lifespan or the induction of any events that might affect a neoplastic response in the highest dose group, the next lower dose level should be examined to the full extent. Microscopic examination in all animals of all groups must be performed of all gross lesions (with a margin of normal tissue). If a significant difference is observed in hyperplastic, pre-neoplastic or neoplastic lesions between the highest dose and control groups, macroscopic examination should be made on that particular organ or tissue of all animals in the study".

47. Para. (v), page 27356, col.3. The last sentence of this para. should be omitted.

48. Para. (vi), page 27356, col.3. Sections (A) and (B) of this para. should be combined to read : "Special examinations. Additional sections should be microscopically examined in all animals of high dose and control groups of the following organs and tissues :"

49. Section 3 of para. (vi), page 27356, col. E, should be extended : "In a dermal study : skin (normal); skin from sites of skin painting including regional lymph node(s)."

50. Para. 772.113-3. Para. (a) (1) (i), page 27357, col.1, should be changed : "The tester should use the rat or another rodent species known to be better comparable to man. If thereare any uncertainties concerning the toxicological profile of the test substance, a test in a second species is recommended. This second species should be a nonrodent species, preferably the dog."

51. Para. (ii), page 27357, col.1. The last sentence of this para. should read : "As part of the study plan, the sponsor must present the rationale for selection of the specific test animals."

52. Para. (ii) (3), page 27357, col. 1. The last sentence of this para. should be omitted.

53. Para. (ii) (4), page 27357, col.1. In the first sentence "50 animals" should be replaced by "25 animals" and "(plus at least 8 additional for clinical laboratory testing)" should be omitted. The last sentence should also be deleted.

54. Para. (ii) (5). Note (ii) on page 27357, col. 2, should be changed to read :"A positive Control Group for particular chemicals is desirable if it can serve as an internal quality control to ascertain whether the test animals are sensitive to, or respond in a predictable manner to, known toxic agents and whether the test strain or species reacts similarly to another strain or species when exposed to the same toxicant."

55. Para.(ii) (6), page 27357, col. 2. The last two sentences should be omitted.

56. Para. (ii) (7), page 27357, col. 2. The second sentence should read : "For gavage, the test substance must be administered for a minimum of 5 days per week ; for feeding, ad libitum; for inhalation exposure, a minimum of 5 days per week, 4 hours per day to the finally achieved actual concentrations for any group on test. "Cf. comment under No. 32.

57. Para.(ii) (8) page 27357, col. 2, should read : "Duration of treatment and observation periods. The tester should administer the test substance to rats for at least 12 months. In studies with nonrodents, the tester should test for at least 6 months." These minimum requirements for testing periods are very often completely sufficient to detect chronic health effects in animal experiments, especially if a toxic dose level is used which shows the full toxicological profile of a substance. As also stated below under n°68.B, geriatric pathology might jeopardize the detection of the genuine toxic properties of a test substanc in experiments lasting too long.

58. Para. (9) (i) (A), page 27357, col. 2. In this para. "must" should be replaced by "should". Furthermore, this para. should be completed as follows :"Exceptions to these requirements are possible, e.g. special inhalation studies with dusts or fibers. The sponsor must, however, justify this decision to the Agency. The sponsor or tester may add additional dose levels at his/her own discretion. If other dose levels are tested, the sponsor must submit the data from any such discretionary levels to the Agency.

59. Para. (9) (i) (B), page 27357, col. 2. The requirement that at the highest dose level of the chronic study animals must die (see Supplementary Information, III. Proposed Test Standards ; Dose selection, p. 27342) cannot be fulfilled if simultaneously a minimum mortality is demanded. In this case the toxicologist's expertise which is based on the results from range-finding tests indicating the toxicological profile of the test substance should be decisive. This section must therefore read : "At the highest dose level clear toxic effects should be demonstrated to give information of the toxicological profile of the test substance."

60. Para. (9) (i) (C), page 27357, col. 3, should be omitted since how to establish NOEL is already mentioned in the first sentence of para. (9) (i), page 27357, col. 2.

61. Para. (9) (i) (D), page 27357, col. 3, should be deleted since it is now included in Para. (9) (i) (A) ; see comment N° 58.

62. Para. (9) (ii), page 27357, col. 3, should be changed : "The tester must conduct an appropriate range finding study to select the dose levels for a chronic study. Data other than from the preliminary study, derived for example from metabolic or pharmacokinetic studies, if available, could be taken into account in selecting dose levels for the chronic study." Cf. comment under No. 35.

63. Para. (9)(iii), page 27357, col. 3. The last word of this para. "submission" should be omitted.

64. Para (b) (1) (i) (A), page 27357, col. 3. As a consequence of comment No. 39 concerning section (2) (i) (B) of para 772.113-2, which also refers to (2) (i) (B) of this

paragraph, a twelve-hour interval of clinical observation is obsolete. Moreover, the requirement of a twelve-hour interval of clinical observation cannot be met if normal daily working hours are observed. Therefore the last two sentences of this para. should be changed: "Clinical observation should be performed at least once a day. At least 4 hours later the animals should be checked to prevent losses due to autolysis and cannibalism. Such losses should not be of such an order as to interfere with full evaluation of the study."

65. Para. (b) (1) (ii), page 27358, col. 1. Clinical laboratory testing.

Most of the determinations performed routinely in animal experiments are semiquantitative. The decision whether, in a chronic study, animals for bleeding should be taken from an additional group or not should be left to the experienced toxicologist planning the study. Therefore the first sentence of this paragraph should read: "The tester should conduct the following determinations on a minimum of eight predesignated rats in each test group." If clinical chemistry methods, well established in humans, are uncritically transferred to experimental animals, fundamental errors may arise. Therefore the following sentence should be added to this paragraph: "Test methods for any laboratory test used have to be referenced."

66. Para. (b) (1) (ii) (A), page 27358, col. 1. Hematology. For reasons of adjustment to possibly shorter testing periods (see Nos. 57 and 71) the first sentence should read: "The tester should conduct the following determinations at least at 3 and 6 months, and every 6 months thereafter: hematocrit, hemoglobin, erythrocyte count, total and differential leukocyte counts, and platelet count." Prothrombin and clotting times should be omitted in this sentence because clotting analysis should not necessarily be performed routinely, but only if considered necessary.

67. Para. (b) (1) (ii) (B), page 27358, col. 1. Blood chemistry. As in para. (A), the first sentence should be changed as follows:" The tester should conduct the following blood and chemistry determinations at least at 3 and 6 months, and every 6 months thereafter: ....". From the list of parameters the following should be omitted:

- "serum lactic dehydrogenase" because of large individual variability in experimental animals;

- "creatinine kinase"; lesions of muscular tissues, e.g. myocardial infarction, are not to be expected in experimental animals, except probably in the pig;

- "creatinine"; only necessary in case of expected kidney function impairment;

- "direct bilirubin"; because of too low plasma concentrations in experimental animals;

- "cholinesterase"; determination should be carried out only in accordance with circumstances as described in FR Vol. 44, July 26, 1979, page 44073, para. 772.112-31 (b) (2) (iii) "Cholinesterase inhibition tests" in subchronic oral dosing studies.

- "triglycerides, albumin, globulin"; should be optional parameters instead of "uric acid, gamma glutamyl transpeptidase, and ornithine, carbomoyltransferase". The latter parameters are either of no value, or there is no experience available in experimental animals.

68. Para. (b) (1) (ii) (C), page 27358, col. 1. Urinalysis. Quantitative determinations in the urine are possible only after catheterization, i.e. a defined volume of urine collected over a certain period of time. Therefore the first sentence

of this paragraph should read: "The tester should conduct the following determinations at least at 3 and 6 months and every 6 months thereafter: ....". Since specific gravity and osmolarity cannot be determined in rodents because the volume of urine is too low, these parameters should not be obligatory.

Urinalysis should be obligatory only in experiments with dogs because in small laboratory animals the value and the credibility of its results are often reduced due to the low volumes attainable.

69. Para. (b) (1) (ii) D) and (E), page 27358, col. 2. Function tests; Residue analysis. These investigations may give useful toxicological information, but it may be scientifically preferable or necessary (radioactive material) to design specific tests for these purposes. Therefore these tests should not be listed and described under this paragraph.

70. For para (2), page 27358, col. 3 and page 27359, col. 1 - 3, "Pathology procedures." the same applies as stated for "Pathology procedures" in para 772.113-2 with only a few exceptions:

- The weighing of spleen and lungs (D) is debatable and should therefore not be prescribed; the same applies to the weighing of adrenals in rodents. Decisions in this respect should be left to the pathologist.

- The examination of (H) aorta and (P) skeletal muscle is justified in this type of experiment. Last sentence of section (v) and section (vi) (A) should also be left unchanged.

71. Para. 772.113-4, page 27359-27367. "Combined chronic effects test standards."

A. In the assessment of non-oncological toxicity we do not accept the necessity for a two-year rat study. By choice of appropriate dose levels it is possible to demonstrate toxicity or to identify the target organs in a study of shorter duration, e.g. 12 months.

B. We are also concerned that interpretation of a two-year chronic toxicity study in the rat will be complicated by geriatric pathology, i.e. the development of spontaneous tumors or circulatory disorders as a result of vascular disease, etc. This problem is overcome by the use of a test of shorter duration, e.g. 12 months.

C. We are not convinced of the scientific desirability of combining in one test (or species) two totally distinct objectives, namely the assessment of carcinogenic potential and of non-oncogenic toxicity.

Therefore we recommend not to set a rigid design for combined studies.

III COMMENTS ON ACUTE TEST STANDARDS

(Reference nos. to comments refer to numbers in this section)

1. Consistency with Pesticide Guidelines : On page 44055, col. 2, the EPA proposes that TSCA test standards should be similar to FIFRA test guidelines. The reduction of the burden on manufacturers which would result from this is welcomed in principle, but ECETOC points out that industrial chemicals differ from the products regulated under FIFRA in that the latter are designed to have biological action and are deliberately spread in use, whereas most industrial chemicals are not designed to have a biological effect and pose a potential risk by the dispersion of only small amounts, as an incidental result of manufacture and use. This fundamental difference should be borne in mind when setting test standards for risk evaluation.

2. Page 44058, col. 2. The maximum losses proposed are unnecessarily stringent. An adequate and more scientific approach would be to require that losses of animals should not be such as to jeopardise the evaluation of the results. There are too many factors involved in a study to permit numerical values to be put on the maximum loss, e.g. if a single animal were lost in an LD_{50} study with 5 animals/group/sex the study would be rendered invalid if EPA'S figures were taken.
The requirement for observation every 12 hours is not justified in an acute study, and would involve an increased number of personnel, on shift. The sponsor should be free to propose and justify the periodicity of observation.

3. Page 44058, col. 3. The EPA suggests that observation of behavioural syndromes should be made. This is too elaborate for an acute study, and we suggest that observations be confined to physical appearance and clinical signs of toxicity.
It is noted that "behavioural toxicity" is a well-defined technical term referring to highly sophisticated methods of investigation which are still under discussion. To avoid confusion, the requirement to observe behavioural syndromes should be omitted, or alternatively a definition adequate for these general studies should be proposed.

4. Page 44058, col 3 ,(2). Histopathological examination is not very useful in an acute study, and should be optional but not obligatory.

5. Page 44058, col 2 (1). The extra benefit to be obtained by the use of a second species or age group in acute studies is very doubtful. An

indication of toxic potential can best be obtained from one well-characterised reference species, and only in special cases (nature of the chemical under test, nature of the effect sought or suspected) the use of a second species or age group would be useful. The use of a second species should be optional.

6. Page 44059, col. 1, (3). The requirement for the use of pathogen-free animals in an acute inhalation study is not justifiable, and is unnecessarily costly. The main purpose of using pathogen-free animals is to ensure long life-time and avoid chronic diseases in chronic studies.

7. Page 44059, col. 1, (4). In discussing whether a sub-chronic study should be for 90 days or 6 months it should be recognised that the former is not solely useful for fixing dose levels in a chronic study but will also demonstrate most toxic effects other than carcinogenicity. Generally a 90-day rat study is of great value on its own, and in the majority of cases will permit reliable risk assessment.
A 6-month study would add little information. Where there is evidence that accumulation is occuring beyond the 90-day period, a 6-month, or longer, study may be justified.

8. Page 44059, col 1, (5). Past dog studies with starting age up to puberty have proved to give valuable information. To set a stringent age limit will lead to logistic problems of animal supply, and choice of the starting age, which must be pre-pubertal, should be left to the qualified scientist.

9. Page 44060, col. 1, section 772.116-2 (1). The recording of individual live foetal weights is technically very difficult and does not give useful information in addition to the remaining tests.
In para.(2), for the increase in the number of rabbits from 12 to 20 the gain in statistical accuracy would not be proportional to the increased cost and effort, and cannot be justified on a cost-benefit basis. Furthermore, most past work has been carried outwith 12 rabbits and has given quite adequate information.

10. Page 44060, col. 2, (3). The Scientific value of the "split study" technique is as yet unproven. EPA should not introduce new techniques until sufficient experience has been accumulated regarding their value and cost.

11. Page 44062, (D). This requirement is of little relevance to acute studies which do not cover the major part of the animals' life span.

12. Page 44062, col 2, (3). The 90% concentration requirement is too stringent, especially when it is considered that analytical data are generally availab only when the substance-mixture is already in use. We propose a figure of 70%, taking into account that the analytically determined concentration is us for calculating the actual intake of test substance per unit of body weight rather than the nominal concentration of substance administered.
Regarding paras (ii) and (iii), the Agency should take into account that analytical methods for determining major degradation products are only exceptionally available, and that the analysis for initial concentration is limited by the physical properties of the carrier (food), by the effectiveness of extraction techniques, and by the analytical precision. In practice a variation of 20% with regard to initial mean concentration, and of 30% with regard to variability between randomly selected samples, should be accepted. Such variations are irrelevant when compared with the variability of biological experiments in quantitative terms.

13. Page 44062, col.3, (4),(ii). The use of feed should be permitted up to its expiry date as given by the supplier. A limit of 90 days should be set only in the absence of such information.

14. Page 44062, col. 3, (4) and (5). There is no scientific justification for using the single standardised diet as given in Appendix B, for acute studies. Similarly, in acute studies the contaminant analysis is not justified.
We would add that the contaminant analysis on each batch is enormously costly, and would be so prolonged as to take up a significant part of the life of the feed before the expiry date.

15. Page 44063, col. 1, (6). In this part much routine work is required to be done by qualified scientists or technical employees who would be over-qualified for the work. This is a waste of scarce resources. For example, looking for cannibalism, autolysis of tissues, and misplacement; weighing of animals, observation of food and water consumption, and taking blood samples do not need to be carried out by qualified technical employees, qualified scientists or veterinarians as defined in the proposals.

16. Page 44063, col. 1, (B). Throughout the proposals for testing for acute toxicity, in the requirements for clinical examination, "signs of toxicity, pharmacologic effects and behavioural changes" should be replaced by "clinical signs of toxicity" - see our comment 3 above.

17. Page 44063, col. 2, (7), (i). In an acute study, gross necropsy need not be carried out by a qualified pathologist, who should simply be available for consultation (i.e. supervision should be "direct", not "personal", as defined).

18. Page 44063, col. 2, (C). Such a detailed examination as is proposed is of doubtful value in an acute study. For example, in a dermal or oral study the examination of the nasal cavity, para-nasal sinuses, spinal cord, and inflation of the lungs, would not normally be justified. We suggest that the paragraph in (C) be replaced by : "The initial physical examination should be left to the judgment of the qualified pathologist".
In para. (ii) the tissue preservation does not require a highly qualified person such as a technical employee or qualified pathologist. The same applies to slide preparation in para. (B), col. 3.
In para. (iv), (A), col. 3, it is not always possible for the same pathologist "to examine and evaluate all microscopic slides....". This requirement should read : "It is preferable that the same pathologist... given species. Appropriate procedures should be adopted to avoid the introduction of bias in observations or professional judgement".

19. Page 44064, col. 2, (3), (i). The molecular structure is not always known. This should read : "....molecular structure if known..."

20. Page 44065, col. 3, Appendix B. There is no obvious justification for requiring that diet for feeding in meal form must be manufactured "by re-grinding pellets". This should be omitted.

21. Page 44066, col. 2, (3), (i). "... showing that no toxicity is evident..." "Toxicity" should read : "mortality" since this is the parameter set for the selection of dose levels in acute studies. See also page 44067,col 1,(4); page 44068, col.1, first word.
In para. (ii), the reference to "95% confidence limits" should be deleted, since analysis of the results will indicate what this is in practice. It cannot be known beforehand how the study is to be designed to give a 95% confidence limit as specified. In any case the confidence limit is not a sufficient criterion for assessing the validity of a study - for example a large confidence interval may result from a shallow slope factor, or if the dose-response curve does not follow a log-probit model.
If a confidence limit is specified it should be broader, and it should be clear whether the interval of 20% means $\pm$ 20 or $\pm$ 10, and whether it relates to the total of males plus females, or to them separately.

22. Page 44066, col. 2, (5). The requirement to receive the same concentration and about the same volume of dosing solution is not possible. A constant volume of solution should be given for the various dose levels.

23. Page 44066, col. 3, (2) Observation. It is adequate to weigh the animals weekly in order to arrive at an indication of a prolonged toxic effect. The requirement to weigh every 3-4 days is unecessary.

24. Page 44067, col. 1, (2). For acute dermal studies the rat is preferred to the albino rabbit. Although the rabbit skin is more easily penetrated than that of the rat, the rat skin is more easily penetrated than that of humans and use of the rat therefore gives an adequate safety factor. One purpose of acute toxicity testing is to compare routes of administration, and it is therefore preferable to maintain the species constant for oral, dermal and inhalation tests.

25. Page 44067, col. 1, (4) and throughout the proposals for dermal studies. The practice of testing abraded skin should be omitted. Abrading cannot be standardised and results from such tests are difficult to interpret. Only intact skin should be tested, as representative of the normal situation.
For this section the same comments apply to the 95% confidence interval as were made in our comment 21 above, especially since it is well known tha the results of dermal toxicity studies have an even greater variability than those from oral studies.

26. Page 44067, col. 2, end of para. (4), (ii). The requirement that "the groups must contain equal numbers of male and female animals" may not be correct if a sex-specific effect is suspected. This phrase should be omitted. The same applies to all other toxicity studies.
In the same paragraph, there is a requirement for the use of 3 dose levels. It is preferable to specify that "enough dose levels should be used to allow for a sound statistical treatment of the results". This is also relevant to page 44066, col. 2, (3), (ii).

27. Page 44067, col. 2, (5). The use of an untreated concurrent control group is not relevant in this test, and should be omitted. Each laboratory will have enough experience with its methods of application to prevent bias

In (b), (1) the requirement to keep the test substance in contact with the skin of at least 10% of the body surface is not possible for highly toxic substances where only small amounts have to be used. For such substances the requirement should be to spread them over as large an area as possible.
The specification of wrapping material should be broadened to permit the use e.g. of aluminium foil. Any impervious, non-reactive material should be permitted.

28. Page 44067, col. 3, (5). This section on histopathology should be omitted because the aim of the acute dermal study is to examine percutaneous systemic toxicity, not effects on the skin.

29. Page 44068, col. 1, (3). The exposure should always be of 4 hour duration (not 1 hour) to allow for adaptation to stress. This is the general practice in Europe. The figure of 5 mg/l, in the preceeding section (2) should relate to a 4 hour exposure - (see also page 44069, col. 2, (6)).
In (2), (ii), on page 44068, the requirements for the number of dose levels, and for equal numbers of males and females in each dose level, should be modified as in our comments n°. 26.

30. Page 44068, col. 1, (5), (i). The use of a concurrent untreated control group is not relevant in this test, as the testing facility will ha enough experience to prevent bias.

31. Page 44068, col. 1, (b), (1), (i). Emphasis is given to whole-body exposure, and alternative techniques are to be considered only "in some cases". We suggest that each technique should be available, according to the circumstances, and that the qualified scientist be free to make the best choice of exposure technique.

32. Page 44068, col. 2, (B). The requirement that chamber concentrations should not vary in a range greater than 30% cannot be achieved in many cases, for technical reasons. This figure will include not only the variations in actual chamber concentration but those associated with the analyses.

33. Page 44068, col. 2, (2), (iii). In acute inhalation studies of 4 hours, variations of temperature and humidity should be 23 $\pm$ 4°C and 30-70%

since in a 4-hour study variations in these ranges are not likely to have a deleterious effect on rats. In a 4 hour-study it may be technically difficult to meet the EPA proposed figures of 24 ± 2°C and 40-60%.

The monitoring of humidity is particularly difficult (availability of measur devices) when exposure is to an aerosol, and allowance must be made for th

The requirement in para (v), (A), to sample for particles when exposure is to a gas or vapour, is of no consequence in a 4-hour study and should be omitted here and from para (B) below. The meaning of the final sentence ("All of the suspended"...) in this para. is not clear.

34. Page 44068, col. 3, (B). The requirement to maintain at least 20% of the particles at 10 microns or less may not be relevant in the light of anticipated exposure during handling, and the physico-chemical properties of the material. These limits should be chosen by the qualified scientist.

For aerosols, analysis once per hour is adequate since size analyses have been carried out frequently during development of the generating system.

35. Page 44068, col. 3, (3). Observation "every 12 hours" - See our comment n° 2. Weekly weighing is adequate - See comment n° 23.

36. Page 44069, col. 1, (A). Microscopic examination of liver and kidney is not justified after a 4-hour study, and examination of the lungs should not be obligatory but should be done only if there is a special reason evident to the qualified scientist.

37. Page 44069, col. 1, (1). The determination of vapour pressure and particulate size should be made only when necessary.

38. Page 44070, col. 2, (3). In the eye examination 24 hours before the test starts, the use of fluorescein should not be obligatory as visual examination is adequate for a pre-examination.

In para. (4), the figure of 9 animals should be reduced to 3, except where additional work is known to be necessary to give an adequate interpretation, because the reaction of the eye of the rabbit is very uniform from animal to animal. Thus for risk evaluation of non- or highly-

irritating materials, 3 animals with un-washed eyes are sufficient for risk evaluation.

In para. (6), (b), washing of the eyes should be optional because it introduces a further variable, is of no relevance to the irritancy of the substance, and is difficult to carry out in a standardised way. The National Academy of Sciences in the U.S. has strongly questioned the use of this procedure.

39. Page 44070, col. 3, (c), (i). Readings of ocular lesions should be made only at up to 72 hours after treatment. Only if effects still persist need further readings be taken at 7 and 14 days after treatment and then weekly. If the pH of the test material is below 3 or above 10 then irritancy may be assumed and the test omitted.

40. Page 44071, col. 1, (4). For the primary dermal irritation test 3 animals is sufficient, unless the response is not uniform enough to permit risk evaluation in which case a further 3 should be tested. The uniformity of skin-response to non-or highly-irritating substances will normally permit a reliable evaluation from 3 animals.

41. Page 44071, col. 2, (b). Omit tests on abraded skin - see comment n° 25. Because the variability between different laboratories and observers is greater than the variability in response of the rabbit skin, in one animal, there is no gain in using more than one patch per animal, i.e. the requiremen for a total of 4 patches should be omitted.

For highly toxic materials there must be an option to use smaller patches and proportionally smaller amounts of the substance. Regarding wrapping material- see comment n°27.

The requirement for 24 hours contact is not relevant to the human situation,and leads to significant changes in the temperature and hydration of the covered skin in contrast to the normal irritation. The contact time should be 4 hours. An open skin irritation test, i.e. without an occlusive patch, is sometimes more meaningful and would be equally valid for regulatory purposes.

In para. (C) the observation and scoring of any irritation should be done weekly, not daily, after the first 72 hours. In para (d).(1), if the pH of the test substance is below 2 or above 12, the material is assumed to be corrosive, and the test could be omitted.

42. Page 44072, col.2, (a).(1). In sub-chronic oral dosing studies the use

of only one species, the rat, should be required. Experience to date shows that the rat has proved adequate to reveal the risks associated with chemicals in the great majority of cases. The probability of gaining significant extra information by testing in a second species is low compared with the extra cost and effort. If the sole purpose of the sub-chronic study is to determine dose levels for a chronic study, then the species to be used in the latter shall be tested in the former. It must be stressed that the subchronic study very often yields sufficient information for reliable hazard evaluation, and should not be regarded as only a range-finding test for a chronic study.

Para. (2). See comment n°8 for age of dogs at start.

Para. (4). Ten animals are sufficient to allow an adequate statistical analysis. The requirement for 20 is excessive.

Para. (5). (ii). The requirement to test non-rodents for 6 months is excessive, as toxic effects will usually be established during 90 days. Where the evidence suggests a need, the test should be carried on for longer.

Para. (6). (i). A sentence should be added, as follows : "Where at a gavage dose level of 10,000 ppm in food or water, or of 1g/kg body weight, no toxic signs are observed, tests at higher dose levels are unecessary since nutritional imbalance will mimic toxic effects". These dose-levels are definitely sufficient for an adequate hazard evaluatio if no adverse effects related to the substance are found.

In para (6).(ii) the requirement for a maximum of 10% fatalities should be replaced by the phrase : "The highest dose level must not lead to a number of deaths which precludes adequate statistical evaluation".

43. Page 44072, col.3, (7), second sentence. As well as oral intubation, administration in drinking water should be allowed.

Para (7), (b), (1), final sentence. A complete opthalmological study should be carried out at the beginning, as well as termination, of a study.

44. Page 44073, col. 1, (ii). Blood chemistry determinations for all sub-chronic studies should be made only at intermediate times (for dogs at e second month only) and at termination, but not at the beginning of dosing. F rodents the determination of serum lactic dehydrogenase, albumin, globulin a direct bilirubin should be omitted as of little value. The final sentence ("The following additional determinations...") should also be omitted for the same reason.

Para (iii). The requirement to carry out 2 intermediate determinations of cholinesterase enzyme activity for plasma and red blood cell is not justifie Determinations should be made at the beginning, at an intermediate time

Para (iv). Urinalysis should be obligatory only for dogs. It gives little useful information from the rat. For dogs, the determination of specific gravity and osmolarity should be omitted since evaporation leads to unreliable results. The final sentence should read : "semi-quantitative" not "quantitative".

45. Page 44073, col. 2, (5). The histopathology examination should be carried out first on the highest dose and control groups. Examination of all animals in intermediate or the lowest dose groups will follow if there is evidence of an effect from laboratory tests, clinical observations and the above histopathology examination.
In para. (ii) the bracket following the word "brain" should read : "(at least 3 levels : forebrain....)"
Para (iii) should begin : "The following organs and tissues, when present, of each test animal...". The bracket following the word "brain" should read : "(at least one longitudinal section)". The word "uterus" should replace "corpus and cervix uteri". Examination of bone (with marrow, and the place from which to take it) in the final sentence should not be prescribed, but left to the discretion of the pathologist.
Para (iii), (B). A sentence should be added : "If no effects are evident in animals exposed to the highest dose level, examination of animals from the lower levels should be omitted".

46. Page 44075, col. 1. The sub-chronic dermal study should be carried out only for chemicals likely to be in repeated contact with human skin. In gener a 21-day test as proposed in the FIFRA guidelines should suffice. There is no justification for a 90-day test if, in use, the chemical is not purposefully applied to human skin.
Para. (4).(ii). For the highest dose level, additionally to the EPA text the following upper level should also be defined.:"the highest dose level should a maximum of 1g/kg body weight, or a dose which produces significant irritancy during the prolonged exposure".
If at this level no substance-related adverse toxic effect is observed, furthe testing is unnecessary since such a result is adequate for hazard evaluation See also comment n° 2 on fatalities.
We disagree with the suggestion (final sentence) of dilution for chemicals producing severe irritancy, and suggest : "The material shall be applied as such. Dilution should not be practiced". Dilution affects penetration in unforeseeable ways and the results would have no relevance to risk assessment for humans.

47. Page 44075, col.2, (5).(b).(1). See previous comment n°27 on "10% of body surface".
In para (2), the saving of half the animals for observation for a further 2 weeks would seriously jeopardise the statistical analysis of the results and should be omitted. There is no scientific justification for a post-treatment observation period in a sub-chronic study, when this is not deemed to be necessary in the corresponding subchronic oral toxicity study.
In para. (3), readings according to Draize should be taken "on the days of application" instead of "daily". The requirement, in the final sentence, for observations every 12 hours has been criticised in comment n° 2.
In para (4), (i), for a study of 21-days' duration as recommended above, haematology determinations need not be carried out at intermediate times.

48. Page 75, col. 3, (ii). The determination of direct bilirubin should be omitted.
In para (iii), it is sufficient to carry out the cholinesterase enzyme activity for plasma and red cell once at the beginning and of the test for a 21-day study, and only at one intermediate time for a 90-day study.

49. Page 76, col. 1, (7), (iii). Weighing of the thyroid (with parathyroid) and the pituitary is not considered useful and should be deleted.
In para. 8, (A), the bracket following "brain" should read : "(at least 3 levels : forebrain...)". The thyroid and parathyroid, not "thyroid (with parathyroid)" should be weighed since in the rabbit these are separate. Instead of "corpus and cervix uteri" only the "uterus" should be specified. Instead of "multiple sections", there should be examined "Representative sections" of treated and untreated skin.

50. Page 44077, col. 2 (a).(1). Use of the rat should not be obligatory for all subchronic inhalation studies. For "must be performed" read "should generally be performed".
Paras (3).(i) and (iii). Previous comments n° 26 and 2 apply to the requirement for dose levels and that concerning 10% fatalities respectively.

51. Page 44077, col.3, (b), (1). The previous comment n° 31 applies to the choice of exposure techniques.

52. Page 44078, co.1, (B). The requirement that the chamber concentrations must not vary by more than 30% is too rigid, and a phrase should be added : "Variations of greater than 30% should be explained".
Para. (iii). The proposed limits for variation in temperature and humidity are acceptable provided that the technical difficulty of maintaining them is recognised by addition of a phrase : "Deviations from the requirement should be explained".
Para. (A). This section on the determination of particles in gases and vapours should be omitted. The laboratory air is filtered, and animals should not be exposed to air which is grossly different from that normally breathed by humans. Without a knowledge of the chemical composition of any particles present, the information from particle determination by weigh would be impossible to evaluate. The exact chemical analysis of undefined particulate matter is practically impossible.

53. Page 44078, col. 2, (3). See previous comments n°16 and 2 concerning "pharmacological signs" and a 12-hour observation frequency.
In para (ii), blood chemistry, see deletions suggested in comment n°44, second paragraph.
In para. (iii), the cholinesterase enzyme activity test should be carried out only once at the beginning and end, and once during the test.

54. Page 44078, col. 3, (6), (iii). The detailed specification concerning lung treatment for the morphological evaluation of the development of emphysema sho be omitted, and only the first sentence of this para left in. This should be left to the judgement of the experienced pathologist.

55. Page 44079, col. 1, (7), (A). The bracket following the word "brain" should read : "(at least one longitudinal section)". "Corpus and cervix uteri" should be replaced by "uterus".

Para (B) should read : "In the intermediate and low dose groups, those organs in which abnormalities were seen in necropsy and histopathology, or where there were signs of alteration during clinical observation or from clinical chemistry."

56. Page 44087, col. 2. In paras (f),(h) and (i) of 772.116-1, see previous comments in Section I (2.2.), and 14 and 15 of this section respectively. In 772.116-2, (a), (1), the choice of species, and how many species should be used, should be left to the qualified scientist. The most extensive experience is with the rat and the rabbit, in that order. They are therefore the most favoured species. In para (3),(i), a positive control group is required. This is not justified since there is no rational choice of control substance, and historical data on control animals are much more valuable.

57. Page 44087, col 3, (5) (i). In teratogenic studies the period of organogenesis should be examined, i.e. treatment should not start before implantation and should be stopped after the major organogenesis is completed. Treatment during the pre-implantation period may influence fertility, e.g. by change of hormonal functions, leading to a decrease in the number of implantations and of material which can be evaluated for a teratogenic effect. Treatment during the foetal phase may lead to functional disorders leading to the death of foetuses, and teratogenic effects which occur during the embryonic phase may possibly not be detected.

Para (6), (i). The specification of "at least 3 dosage levels" needs qualification.When a level of 1000 mg/kg body weight/day, or 10,000 ppm in feed, cause no adverse effects on the foetuses, testing at higher dose levels is irrelevant. The same is true when a dose level causes maternal toxicity but without impairment of the foetuses. Thus in certain cases a control plus 1 dose level is sufficient.

In para 6, (v), the following should be added "... test substance administration, or may be adjusted to changes in body weight at the discretion of the qualified scientist".

Generally the test in 772.116-2 is carried out to detect embryotoxic (includi teratogenic) effects. Thus "teratogenic" should be replaced by "embryotoxic".

58. Page 44089, col.2, second line. See previous comment n° 2 on "10% fatalities"

59. Page 44089, col.3, (4), (i). This should begin : "all animals used to produce the F_2 generation must be subjected...". We do not understand the reason for suggesting "10 males and 25 females".

The histopathology should be performed first on the control and high dose groups, and then on the lower dose groups only where the evidence from the high dose group indicates a necessity. Since in a reproductive study adverse effects on reproduction are sought, only the gonads should primarily

examined histopathologically. Additional histopathology should be carried out only on target organs as revealed by necropsy or clinical symptoms.

60. Page 44092, col 1 (§772.119-1(b) (1) through (6). The general metabolism studies outlined in these proposals reveal a strong influence of a thinking in terms of pharmaceuticals. The pharmacokinetic approach required appears inappropriate in the field of general chemicals, both from a safety and residue standpoint. When general chemicals are studied in laboratory animals, such an approach specificall stressing the importance of absorption is unnecessary. This is especially so because not absorption as such but i consequences with respect to toxicology and organ residues are of relevance. These latter two parameters, however, need for their proper determination neither an intravenous application study nor a detailed knowledge of the kinetics of blood residues.

The purpose of the metabolism studies required is adequately and sufficiently defined in chapters (1 (2), (4) and (6) of this section. According to the model character of those studies their results should be evaluated mainly in qualitative terms.

61. Page 44092, col 1, (e). For metabolic studies, detailed procedures should not be laid down because such studies have to proceed in a step-wise manner according to the results obtained at each stage. Radioactive materials are generally used in metabolism studies, but for some purposes the unlabell substance is adequate, e.g. measurement of the half-life of the parent compound in body fluids. The decision whether to use a labelled or unlabelled substance should be left to the qualified scientist.

Labelling with ^{3}H by the Wilzbach procedure should not be excluded since it can be useful under special circumstances. The specifi cation that"some animals must receive repetitive doses of non-radioactivel labelled test substance" should be modified since, in some cases, single dosing gives adequate information.

62. Page 44092, col 2 (e) (2) (iii). Using experimental data from a large number of balance studies performed in the past, a statistical comparison of the alternative use of 5 animals as required or 2 animals as proposed in these comments has been made. It revealed that the consequences of the larger number of animals would only be the reduction of the standard error of the means by a factor of less than 2. This insignificant improvement of the precision does not justify the disproportionate effort required.

63. Page 44092,col.2 (e) (3).See comments under (f) (1) (i) and (iii) Provision for routes of administration (dermal and inhalation) other than oral should be made.

64. Page 44092, col 2(e) (4). Low dose (group B animals): The dose reflect the potential environmental concentration of the chemical, including a reasonable safety factor. An average dose of 0.5 mg/kg body weight corresponding to about 5 ppm in food generally will meet this requirement.

High dose (group D animals) : The dose should give an indicatio of the metabolic capacity of the experimental animal for the test compound. Generally, dose levels already causing toxic effects are accompanied by a disturbed metabolism. Under those conditions useful information would probably not be obtained. It would even be questionable whether it could be used to assist in the evaluation of accidental poisoning. We are of the opinion that 25 mg/kg, or one third of the oral LD_{50} whichever is lower, would sufficiently take into account potential high level exposure. In addition, a fifty fold dose range would certainly allow recognition of any significant dose dependency of metabolism.

65. Page 44092,col 2, (f) 1 (i). The intravenous application study proposed to yield short-term pharmacokinetic information on the absorption of a chemical substance is not meaningful Before any animal metabolism study is started, standard toxicity data of the compound under study such as oral, inhalatory, dermal and intraperitoneal LD_{50}'s are already at hand. From this data and from the rate and extent of excretion of radioactivity via urine and expired air after oral application of the compound, a reasonable evaluation of its absorption potential can be made.

66. Page 44092,col 2 (f) 1 (iii). Multiple application metabolism experiments essentially aim at the evaluation of the long-term organ-specific accumulation potential of the test compound rather than relating its absorption to the duration of the exposure of the animal. These studies should beperformed only when the single-dose balance studies show significant organ residues as outlined below in the section "Conclusions" : (§772.119.1) under Evaluation.

In addition, direct determination of the build-up and depletion phases of the plateau of radioactivity in individual organs and tissues (i.e. dynamic equilibrium) is a more suitable measure of the effect of multiple dosing than determination of blood levels directly after application of a labelled dose at the end of a non-labelled dosing period and of the organ residues remaining 7 days thereafter.

67. Page 44092,col 2 (f) 2. The requirement of this section will have the consequence that any general metabolism study will be terminated after 7 days, not leaving the alternative of 95 % excretion, as the excretion data cannot be determined and computed continuously during the experimental period with a justifiable effort.

68. Page 44092, col 3 (f) (3)(i)(B). Direct correlation of the magnitude of tissue residues to pathological phenomena in these tissues is difficult to make as, generally, the concentrati of the chemical needed for an effect at the site of action is not known. In addition, metabolism studies generally are run simultaneously with long-term toxicology studies only at the end of which possible pathological effects are disclosed. It is only then that it should be discussed whether additional information from metabolism studies could contribute to an understanding of pathological effects. The part of the sentence "any tissue or organ which demonstrated pathology" should therefore be omitted. This also applies to paragraph 772.119 -1(g)(1)(i), page 44093, col 1.

69. Page 44092, col 3 (f) (3) (i) (C). The requirement to determine the extractibility of residual organ radioactivity in all g (with a view to tissue binding) is meaningless unless significant organ residues have been found in the single, low-dose experiment (group B).

70. Page 44092, col 3 (f) (3) (ii): see chapter 3 of Conclusion par. 772.119-1 below.

71. Page 44092, col 3 (f) (4) (i) and (ii). Comments on the significance of absorption from the digestive tract in metabolism studies with general chemicals has been outlined in the comment on section (b)(1) through (6), page 44092, col

With respect to the rate of absorption, the experimental approach required in this section cannot reasonably be followed because the frequent blood samplings schedules would render a balance of radioactivity practically impossible.

With respect to the extent of absorption, the required procedu i.e. the comparison of excretion data after intravenous and oral application, may lead to ambiguous results. The patterns of metabolites in blood after the two routes of application need not be identical as the chemical and biochemical activit of the intestinal tract (including microflora and mucosa) may transform the parent chemical partially or even completely pric to absorption. Compared to a parent chemical intravenously injected, this transformation could result in derivatives with different distribution, further metabolism and/or excretion patterns.

72. Page 44093, col 3(f) (4)(iii). This requirement is meaningful only when the prerequisite mentioned in above comment under 1(f)(3)(i)(C) page 44092, col 3 is given.

73. Page 44093, col 1 (g) (1)(ii) and (iii). According to our comments on section (b)(1) through (6), page 44092, col 1. and section (f)(1)(i), page 44092, col 2 these sections are no longer relevant.

74. Page 44093, col 1-2, (g) (2) (i) through (vi). According to the above comments, parts (i), (ii), (v) and (vi) of this section are no longer needed.

With respect to part (iii), the excretion of xenobiotics generally follows a multiple phase pattern which is not described correctly by a single "beta phase t 1/2" value. The time intervals needed for 50 % and 90 % excretion of the radiolabelled dose are an adequate basis for the evaluation or comparison of the excretion behaviour of compounds.

With respect to part (iv), the potential for and the organ specificity of bioaccumulation of a xenobiotic will be recognized by the residual organ radioactivity at the end of the single-dose balance study. An accurate description of the bioaccumulation process can be deduced from the multiple-dose experiment recommended in the comment on section (f) (1) (iii), page 44092, col 2.

75. Conclusions. §772.119-1 (our remarks 60 to 74)

Summarizing the consequences of the above comments the following set of experiments is considered necessary to provide the information required under sections 772.119 -1 (b) (1), (2), (4) and (6) of these proposed rules :

1. Oral, single-dose balance study

- Substances to be tested : analytically pure, radiolabelled active ingredient.
- Animals : 2 young, adult rats per test group, preferably of the strain used in long-term toxicology; individual housing.
- Test groups and dosage :

  . Low-dose } males
  } females
  0.5 mg/kg or 1/3 of the oral LD_{50} whichever is lower.

  . high-dose } males
  } females
  25 mg/kg or 1/3 of the oral LD_{50} whichever is lower.

  . total number of animals : 8

- Duration of test : 6 days
- Analysis : percent excretion of radioactivity in urine, faeces and, if necessary, expired air ; residual radioactivity in the organs and tissues required under (f)(3)(i)(B) including blood. Qualitative comparison of metabol patterns from low- and high-dose study.

- Calculations : total recovery, time intervals needed for 50% and, if possible, 90 % excretion ; limits of detection and quantitation for residual organ radioactivity.

- Evaluation: When the single dose balance studies indicate significant organ residues as a consequence of

  - partitioning in lipophilic tissues
  - reactivity with body constituents in specific organ(s)
  - re-use of fragments of the compound under study in the endogeneous metabolism of the test animal,

  then the following multiple-dose organ plateau study, or a study specifically designed to elucidate the formation and nature of those residues, must be considered.

2. Oral, multiple-dose, organ plateau study:

- Substances to be tested: as under 1

- Animals : Male, young, adult rats as under 1 ; females in cases where the residues defined under 1. would favour this sex ; 2 animals per time interval.

- Dosage and duration of test : 0.5 mg/kg daily until a plateau of radioactivity is established in the organs required under (f)(3)(i)(B) page 44092, col 3, or 14 days whichever co first. After discontinuation of dosing, depletion is followed until 0.1 ppm radioactivity in the relevant organs is reached or until 5 days whichever comes first.

- Analysis : concentration of radioactivity in the relevant organs as dependent on time, covering the phases of build-up of the plateau and of depletion of the radioactivity Extractability of residues in individual organs to check possible tissue binding.

- Calculation: time interval between start of dosing and reaching the plateau in the individual organs : height of plateau compared to dose daily applied; depletion interval and/or ratio of concentration of radioactivity at plateau and at the end of depletion; if appropriate, statement that plateau could not be reached within 2 weeks (chemical accumulates).

3. Metabolite Identification

Radioactivity excreted in urine and faeces of the oral high-dose application is used for the identification of significant metabolites. In cases where qualitatively different metabolite patterns are obtained after low- and high-dose applications, identification after low-dose application is also necessary.

Generally, it is felt that metabolite identification should aim at the establishment of the metabolic pathways in order to evaluate possible correlations of the metabolites and their theoretical intermediates with toxicology.

4. Additional metabolism studies

If needed, the nature and extent of additional, more specific studies should be discussed between the applicant and the agency.
