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CC/08/11

**COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD,
CONSUMER PRODUCTS AND THE ENVIRONMENT**

EXCESS MORTALITY IN TWO-YEAR RODENT CARCINOGENICITY STUDIES

Background

1. The OECD Test Guidelines 451 (Chronic Carcinogenicity Study in Rodents), TG 452 (Long-Term Chronic Toxicity Study in Rodents by Oral Administration) and TG 453 (Combined Chronic Toxicity/Carcinogenicity Studies) are being revised to bring them into line with current scientific knowledge and good practice. A Guidance Document is being produced alongside these revised Test Guidelines and the COC considered the Chapter on dose selection for chronic animal bioassays at its meeting on 10 April 2008.
2. One of the issues under consideration for the revision of the Test Guidelines is the duration of the studies, in particular dealing with mortality before the scheduled termination. The relevant section of the current (1981) version of TG 451 "Carcinogenicity studies" is cited below:

"Duration of study

It is necessary that the duration of a carcinogenicity test comprise the majority of the normal life span of the animals to be used. It has been suggested that the duration of the study should be for the entire lifetime of all animals. However, a few animals may greatly exceed the average lifetime, and the duration of the study may be unnecessarily extended and complicate the conduct and evaluation of the study. Rather, a finite period covering the majority of the expected life span of the strain is preferred since the probability is high that, for the great majority of chemicals, induced tumours will occur within such an observation period.

The following guidelines are recommended:

- (a) Generally, the termination of the study should be at 18 months for mice and hamsters and 24 months for rats; however, for certain strains of animals with greater longevity and/or low spontaneous tumour rate, termination should be at 24 months for mice and hamsters and at 30 months for rats.*
- (b) However, termination of the study is acceptable when the number of survivors of the lower doses or control group reaches 25 percent. For the purpose of terminating the study in which there is an apparent sex difference in response,*

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each sex should be considered a separate study. In the case where only the high dose group dies prematurely for obvious reasons of toxicity, this should not trigger termination.

In order for a negative test to be acceptable, it should meet the following criteria:

(1) No more than 10 percent of any group is lost due to autolysis, cannibalism, or management problems.

(2) Survival of all groups is no less than 50 percent at 18 months for mice and hamsters and at 24 months for rats.

3. At the OECD meeting held in Washington in February 2008, the FDA indicated that they wanted the normal duration changed to 2 years for mice as well as rats, as mouse strains were now used that survived for 2 years.

4. The FDA also does not recommend terminating a study because the survival rate in controls falls below 25%.

5. The FDA strongly disagrees with the statement that "Survival in each group in the study should be no less than 50 per cent at 18 months for mice and hamsters and 24 months for rats". They consider that a carcinogenicity study still can be quite acceptable even if the entire high dose group dies before the end of the study, as the lower dose groups still may be used for the evaluation. The FDA would, however, recommend removing a group from a study, stopping of dosing of that group, or dropping of the dose for that group if they were notified of survival problems.

6. These issues were brought to the meeting of National Coordinators in April 2008 but were not resolved. Furthermore, no agreement has been reached on what constitutes the high dose that would be acceptable to all regulatory authorities if the concept of the Maximum Tolerated Dose were removed. The issues of the maximum dose and survival are clearly linked. For genotoxic carcinogens, it is assumed that there is no threshold. Therefore, in order to detect genotoxic agents of low potency, high doses are used to avoid the large numbers of test animals that would be needed for adequate sensitivity at low doses. However, such high doses may be irrelevant for non-genotoxic carcinogenesis and may lead to high mortality from non-tumour-related causes. Any study mortality has a detrimental effect on the power of the assay for a dose/tumour response analysis. There is therefore a need to balance these opposing requirements.

Published Proposals for dealing with mortality

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7. The attached paper by Roth et al (2007) (Appendix 1) considers the impacts of various patterns of differential or excess mortality on the biological and statistical interpretation of 2-year rodent carcinogenicity studies. The paper considers 4 different situations:

- increased mortality in only the high dose group
- increased mortality in the high dose group and other dose groups but not the controls
- increased mortality in one of the dosed groups but not the high dose or control groups
- increased mortality in the control group or controls and one or more dosed groups

8. The paper considers the effect of different design modifications (dose reduction and/or termination of one or more groups) and modifications to the analysis.

9. The paper also recommends consultation with the FDA on the acceptability of the approach selected. However, this is not satisfactory for a carcinogenicity test, which should be acceptable worldwide to prevent repetition of studies designed for individual regulatory authorities. For this reason, it is important that the OECD Test Guideline gives a clear recommendation, as the OECD member countries are obliged to accept any Guideline-compliant study under the Mutual Acceptance of Data agreement.

COC Advice sought

10. The COC is invited to:

- i). comment on the paper by Roth et al (2007)
- ii). indicate whether they agree with the recommendation, if there is excess mortality in the high dose group, to amend the protocol to:

a) reduce the dose in the high dose group to a level \geq mid dose

AND IF (a) FAILS to reduce the mortality then:

b) EITHER terminate the high dose group for humane reasons without histology if it is clear that the deaths are not tumour-related

c) OR reduce the dose in the high dose group to a level $<$ mid dose

AND IF (a), (b) and (c) FAIL OR ARE NOT APPLICABLE

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- d) early termination of the high dose group with histology for estimating tumour incidence
 - e) early termination of the high dose and some controls or all groups depending on the timing of the deaths
- iii). indicate whether they agree with the recommendation, if there is excess mortality in the high dose group, to amend the analysis to:
- f) inclusion or exclusion of the high dose group data in a trend test depending on whether the high dose exceed the MTD
 - g) OR a two-group comparison of high dose versus controls
 - h) AND an analysis of all deaths in the absence of fatal tumours to determine whether the high dose exceeds the MTD (ie was unacceptably high)
- iv). indicate whether they agree with the recommendation, if there is excess mortality in the high dose group and other treated groups, to amend the protocol and analysis as in (ii) and (iii) above.
- v). indicate whether they agree with the recommendation, if there is excess mortality in treated groups other than the high dose group, to run the study to completion.
- vi). indicate whether they agree with the recommendation, if there is excess mortality in the controls only or controls and one or more treated groups, to continue the study only if the number of surviving animals is similar across the groups.
- vii). advise whether the wording in the “Duration of Study” section of the 1981 Test Guideline 451 should be revised, and if so how. The COC is reminded that if specific requirements on procedures to deal with excess mortality are included in the Test Guideline, they become obligatory under the Mutual Acceptance of Data agreement.
- viii). advise whether recommendations on dealing with excess mortality should be added to the Guidance Document being drafted to accompany the revised versions of the Test guidelines. Such recommendations would not be obligatory under Mutual Acceptable of Data.
- ix). advise whether normal duration of carcinogenicity studies in mice should be revised to 2 years, remain as it is, or allow flexibility (with the inherent danger of unacceptability, to the US FDA, of study duration < 2 y).

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Secretariat

June 2008

Reference

Roth A, Kadyszewski E, Geffray B, Paulissen J & Weaver RJ (2007) *Toxicologic Pathology* 35 1040-1043

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Appendix 1 to CC/08/11

EXCESS MORTALITY IN TWO-YEAR RODENT CARCINOGENICITY STUDIES

Roth A, Kadyszewski E, Geffray B, Paulissen J & Weaver RJ (2007) *Toxicologic Pathology* 35 1040-1043

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