

MUT/07/08

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

FURTHER CONSIDERATION OF APPROACHES TO RANKING IN-VIVO MUTAGENS: THE LOWEST EFFECTIVE DOSE (LED FOR IN VIVO GENTOXICITY); A POSSIBLE APPROACH TO MUTAGEN POTENCY RANKING

Background

1. The COM and COC have previously discussed approaches to wider dissemination of their advice regarding Comparative Risk Assessment. The COC had agreed in principle to use the Margin of Exposure (MOE) as an additional tool to aid in risk communication on genotoxic carcinogens at its November 2006 meeting. However, there had been no final agreement on the banding approach which could be used to rank and communicate risk and on the descriptive terms that should be used for the different degrees of risk in each band. The COM discussed a pragmatic approach to ranking in-vivo mutagens, which did not have carcinogenicity data, based on the suggested approach of the The Lowest Effective Dose (LED) developed by Sanner and Dybing 2005.
2. It was acknowledged that there were problems with LED approach suggested by Sanner and Dybing and there were arguments against the proposal i.e. it would involve use of data from a wide number of end points with varying sensitivity, that the approach depended upon available published data, and there would be a publication bias. These were considered to be valid criticisms, but the desired outcome was broad categories which might be helpful for pragmatic risk ranking. The COM agreed that some further consideration could be undertaken using chemicals categorised by IARC as group 1 and 2A carcinogens. Members also asked for a consideration of chemicals within structural classes.
3. This paper outlines the further work undertaken by the secretariat with regard to the development of a generic approach to ranking in-vivo mutagens. One outcome has been that it is not possible to derive LED potency boundaries for any in-vivo tests other than the rodent micronucleus tests.

Introduction to current review paper

4. An important consideration in reviewing in-vivo mutagenicity data from chemicals within databases is the process by which chemicals were selected for the databases and the comparability of mutagenicity data for the chemicals within the database. For the IARC database, there 36 chemicals in group 1 and 49 in group 2A. (noting that groups such as inorganic cadmium or

nickel are counted singly). These numbers exclude chemical mixtures, exposure circumstances and combination medicine treatments and radiation sources). The mutagenicity data for these chemicals were briefly reviewed in the monographs but for most chemicals it was not possible to derive LED estimations without going back to the source references. There was insufficient resources to do this. However a published evaluation of rodent micronucleus tests for IARC carcinogens was obtained which is considered in detail below (see paragraphs 20-22 below).

The secretariat also considered whether relevant data could be easily obtained from the EU classifications for category 2 and 3 mutagens. These chemicals can be identified through searching for appropriate risk phrases on the ECB internet site. (<http://ecb.jrc.it/classification-labelling/>)

However in only a few cases could a sufficiently detailed summary of data be readily obtainable which would allow an LED value to be derived.

Further consideration of EU classified mutagens would extend the COM evaluation of a pragmatic approach to in-vivo mutagenicity potency ranking but it is unlikely there will be sufficient resources to do this work.

5. The secretariat identified a published evaluation of rodent micronucleus tests undertaken as part of the EPA Gene Tox program during the 1980s and 1990s and it is suggested that these data can be used to guide discussions.

LED values published by EPA Gene Tox Program

6. The published information has been derived from Mavournin KH et al (Mutation Research, 239, 29-80, 1990, see Annex 1 for full paper). This relates to 415 chemicals and includes many considered by IARC up to the publication date. The EPA internet site lists that data have been derived under this program for 506 chemicals, but it is evident that not all the current data are in the public domain or readily accessible for the derivation of LED values.

7. A further advantage of using data from the EPA Gene Tox program is that the data were evaluated by a standardised approach and the LED values allocated by the authors after review of the reports for each chemical. However as noted below for some chemicals there is a wide range of reported LED values. The default has been to select the lowest value. The proposal for consideration a potential environmental contaminant which is not included in the dataset would be to select the lowest numerical value, and to only disregard this value when the study was considered to be inadequate. In most cases this would be unlikely as there would need to be convincing data and arguments to overrule a clear positive result.

8. A brief evaluation of the intraperitoneal dosing studies in mice was undertaken using the lowest published LED estimate. This route of administration was chosen as most data in the publication refer to intraperitoneal

studies. Complete adsorption is considered to be a pragmatic assumption for such studies. Applying some arbitrary dose limits for subdividing chemicals it is possible to provide some preliminary ranking of the chemicals in the data base.

Initial consideration of potency bands

High potency

9. Thus using ≤ 2 mg/kg bw for high potency, ≤ 200 mg/kg for medium potency and >200 mg/kg bw for low potency. The possible use of alternative descriptor words for potency groups is discussed below. The low potency boundary is set on the limit dose. The medium and upper boundaries can only be set on the properties of the chemicals which fall into the groups. The general advice for subdividing potency carcinogens (essentially based on evaluation of TD50 values separated by a factor of 10) is unlikely apply for in-vivo mutagen ranking. Some further consideration is given below as to how the proposed approach would be influenced by molecular weight considerations of the chemicals included in the database, chemicals where the available data suggest a wide range of potency estimates and potential approaches to inclusion of data from studies derived using the oral route of exposure. Compounds such as toluene which are not considered to be mutagenic in-vivo have been excluded.

Information on high potency chemicals (i.p. MN assay in mice)

Chemical	General information
Acranil	Anti viral, interferon like substance.
Aflatoxin B1	Food/environmental contaminant
Aminopterin	Folic acid derivative
Cannabinol	Constituent of cannabis
Cannabidiol	Constituent of cannabis
Cis-platin	Cytotoxic chemotherapeutic agent
Colchicine	Medicinal use in gout
Cytosine arabinoside	Cytotoxic chemotherapeutic agent
Dimethyl carbamoyl chloride	Chemical intermediate
5,5-diphenylhydantoin. Sodium	anticonvulsant
Hexamethylphosphoramide	Polymer and selective solvent, stabiliser
Hippeastidine	Plant extract medicinal uses
Mechlorethamine	Cytotoxic chemotherapeutic agent
Mitomycin C	Cytotoxic chemotherapeutic agent
Monocrotophos	OP pesticide
Phosphamidon	Op pesticide
Sibiromycin	Antitumour antibiotic
Sodium meta-arsenite	Environmental contaminant
Triethylenemelamine	Environmental contaminant

3,4,5,-Trihydroxy-3,6,7,8-tetramethoxyflavone	Antifungal flavenoid
Vinblastine	Cytotoxic chemotherapeutic agent
Vinblastine sulphate	Cytotoxic chemotherapeutic agent
Vincristine	Cytotoxic chemotherapeutic agent
Vincristine sulphate	Cytotoxic chemotherapeutic agent

10. The 24 chemicals identified represent a range of cancer cytotoxic medicines, some industrial chemicals a small number of the most highly regulated environmental contaminants and some well known high potency in-vivo mutagens.

11. Aflatoxin B1 is an interesting example of a borderline compound, since with a published LED value of 2.5 mg/kg bw it is borderline with regard to high and medium potency. It can be argued it would be prudent to assign aflatoxin B1 to the high potency group. It is notable that the only other i.p LED value for this compound (5 mg/kg bw) is also close to the arbitrary cut off of 5 mg/kg bw. There are no guidelines for selecting numerical LED values for considering a higher potency grouping. It is essentially a case-by case decision. In this instance the consistency of available in-vivo mutagenicity data would be important and possibly the relatively high molecular weight of aflatoxin B1 compared to low molecular weight alkylating agents (see section on molar doses below).

Medium potency

12. The brief evaluation of the EPA Gene Tox publication identifies approximately 90 chemicals with intraperitoneal LED mouse values of $>2\leq 200$ mg/kg bw. The larger number of compounds in this group compared to the high potency group may simply reflect the chemical selection for testing rather than a true distribution of mutagenic potency. A full listing of the chemicals in this group has not been provided in the cover paper, but the group includes some well known in-vivo mutagens.

13. This group includes some well known in-vivo mutagens used as positive controls in mutagenicity assays and a number of well know genotoxic carcinogens. Examples of in-vivo mutagens used in mutagenicity assays includes cyclophosphamide, EMS, ethylnitrosourea, and MMS. It is notable that there is a tendency for the range of LED values for the group of in-vivo mutagens to be relatively small (e.g. EMS 100-200 mg/kg bw, and MMS 20-50 mg/kg bw). This may simply reflect dose selection based on the large amount of information available on these compounds. The genotoxic carcinogens include 2-

acetylaminofluorene, acrylonitrile, benzidine, benzo(a)pyrene, 1,3-butadiene, 7,12 DMBA, DMN, urethane and vinyl toluene.

14. Some further consideration comparing data for low molecular weight alkylating agents such as MMS/EMS with higher molecular weight compounds which form bulky adduct such as benzo(a)pyrene is given below under molecular weight considerations.

Low potency

15. The brief evaluation of the EPA Gene Tox publication identified 11 chemicals where the i.p LED mouse values were >200 mg/kg bw. N-butylglycidyl ether, ethylacrylate, 4-hydroxymethylbiphenyl, 4-methylphenylenediamine phenacetin, styrene were reported with LED values of >200 but <1000 mg/kg bw. A small number of compounds were documented with values of ≥ 1000 mg/kg bw which included 4,4-diamnioterphenyl, ethionine, trimethylphosphate, tris (2,3-dibromopropyl phosphate) and vinyl acetate.

16. This group does include benzene based solely on i.p mouse LED data. The data presented in the EPA gene tox publication for i.p mouse MN tests shows the lowest LED to be 440 mg/kg bw. It is possible that the subcutaneous mouse study where 6daily treatments of 88 mg/kg bw to suggest inclusion in the medium potency group. However the data for benzene does highlight the problems of using arbitrary cut off limits and the need for a thorough review of all data for each compound. Additionally the proposals outlined in this paper for ranking would apply to compounds for which there were no carcinogenicity data and in many instances for compounds not included in regulatory approval schemes or marketed at tonnages where there might be little probability that carcinogenicity data would become available.

Consideration of molecular weight

17. The COM/COC have previously considered that potency values should best be expressed in term of molar dosages. However hazard classification dosages, limit dosages used in mutagenicity studies and potency cut off dosages for carcinogens are all expressed in mg/kg applied dose. There is essentially no history of reporting dosages in terms of molar dosages. One possible approach which could be used to assess whether the molecular weight of a compound should influence a decision to allocate to one or another potency group is to examine the range of molecular weights for in-vivo mutagens. Low molecular weight alkylating agents such as MMS (Mw 110) could be compared to higher molecular weight in-vivo mutagens such as (7,12 DMBA (256), B(a)P (252), aflatoxin B1 (312)). This gives an approximate ratio of 3 fold. Thus if an in vivo mutagen gives an i.p potency value in mouse MN assay within 3 fold above a

potency cut off value then some consideration of molecular weight should be undertaken.

Thus the values for considering molecular weight would be 6 mg/kg for inclusion in the high potency group, and 600 mg/kg bw for inclusion in the medium potency group. It is possible that members could specify higher weight molecular in-vivo mutagens and the ratio compared to low molecular weight mutagens such as MMS would be larger. It is not proposed to express potency values in terms of molar doses but to acknowledge molecular weight when making decisions on which group to allocate compounds in.

Consideration of oral mouse micronucleus assays.

18. Many mouse micronucleus assays currently undertaken use the oral route of administration. The amount of test material absorbed reaching the bone marrow as active metabolite has been a particular difficulty in assessing these studies. Comparatively few data on oral and i.p studies are available in the EPA Gene Tox publication and there are insufficient data to make estimates of the potency ranges for oral administration. There are examples of very similar estimated potencies using oral and i.p studies in mice (e.g DMN, MNNG, cyclophosphamide) and other examples where the evidence as presented suggests much lower potency via the oral route e.g 7,12-DMBA, and other examples where there is considerable overlap between the oral and i.p estimates of potency e.g B(a)P. One pragmatic approach would be to use an estimation of percentage absorption if this were available to adjust the estimated oral LED value so an assessment of potency could be achieved using the proposed banding. This approach would be equivalent to that used in the derivation of Acceptable Operator Exposure Levels (AOELs) used in risk assessment of operator exposure to pesticides where the NOAEL data for risk assessment are derived from oral studies in experimental animals. It is noted that there is no inclusion for extent of first pass metabolism in this approach but it would represent one possible way to include oral studies in the proposed scheme.

Consideration of 6th collaborative study by Collaborative Study Group on Micronucleus Test (CSGMT) and Japanese Environmental Mutagen Society (JMS)

19. The 6th collaborative study investigated the results from approximately 100 mouse micronucleus assays (predominantly undertaken by intraperitoneal dosing) for selected chemicals which had been evaluated for potential carcinogenicity by IARC. Sufficient data can be accessed to expand the

database of i.p LED values for a number of compounds for which published data are not available from the EPA gene Tox program. At the present time this additional work has not been completed. A copy of the sections of the paper reporting methods, results, and interpretation is appended as Annex 2. (it is noted the full published paper is approximately 120 pages in length.)

20. The focus of the consideration of the CSGMT/JEMS evaluation was to consider how the results of the mouse micronucleus test correlated, if at all, with the outcome of IARC evaluations. Overall a rough correlation of increasing percentage of positive chemicals in the mouse micronucleus test with increasing assessment of potential carcinogenic hazard to humans was reported. For the purposes of this evaluation, the main consideration relates to the method for reporting potency. The authors use an arbitrary combination of 1% MNPCEs (10‰, 10/1000) at a dose of 100 mg/kg bw or less. From table 16 of the paper, there is a range of dose values reported for a treatment related increases of 1% or more. From 0.5 mg/kg bw (nitrogen mustard) to 500 mg/kg bw for nitrosomorpholine. If these dose levels represented the LED value (which is not stated) they would span the high and low potency values suggested above.

21. It is proposed that an additional check to considering molecular weight and oral dosing when evaluating LED values, would be to include the response of 1% MN PCEs at the LED value. If the compound induced 1% or more MNPCEs at the LED, then consideration should be given to whether a higher potency ranking was appropriate. This would be a case by case approach based on all the available data for the compound (including the LED value, any evidence for a steep dose response, evidence for significant absorption).

COM discussion: Overall approach to in-vivo mutagen ranking

22. As a guide the following steps are recommended. Members are asked to consider each step and the overall process.

a. consider all available data. If carcinogenicity data available then rank according to T25 etc. if no carcinogenicity data, determine i.p LED value from most appropriate mouse micronucleus test (usually lowest LED value, unless study considered inadequate). Pragmatic definitions of potency bands for i.p mouse MN assays, ≤ 2 mg/kg bw (high), 2- ≤ 200 mg/kg bw (medium) and >200 mg/kg (low)

b. if oral study available (but no i.p study), adapt estimate for LED according to percentage absorption. For compounds with not oral absorption data, consider using algorithm for estimating approximate oral absorption. (one option is to derive default oral absorption figures which are over estimates)

- c. consider if LED value is close to a potency boundary (values of 6 mg/kg bw for high potency, and 600 mg/kg bw for low potency have been suggested), should the compound be placed in a higher potency grouping. (Derive ratio for Mw compared to a low molecular mutagen e.g. MMS (ca 100)).
- d. Consider percent induction of MN PCES at the LED value. If above 15, consider placing in a higher potency group.

Information on ranking in structural series

23. A limited amount of published literature was retrieved with data for in-vivo mutagenicity studies with a number of compounds in the same structural groups. The data tended to be derived from experimental approaches which are unlikely to be used in screening studies for unknown compounds which limits the applicability of the data more generally and there were no specific studies using the mouse micronucleus test retrieved (other than studies of benzimidazoles (e.g Barale R et al Mutation Research, 300, 15-28, 1993 previously seen by COM). Members will also be aware of the work by Bieje B and Ashby J (Carcinogenesis, 6, 611-615, 1985) which reported contrasting potency of four azo compounds in-vitro in Salmonella typhimurium mutagenicity tests compared to in-vivo rat liver UDS assays (Carcinogenesis, 6, 611-615, 1985).

24. Thus Nishikawa T and colleagues (Mutation Research, 588, 58-63, 2005) reported skin MN assays in hairless mice for a number of PAHs which the authors considered showed a correlation between mutagenicity and carcinogenicity. Brambilla G et al (Carcinogenesis, 2, 425-429, 1981) reported a quantitative correlation among DNA damaging potency of six N-nitroso compounds (using alkali elution following oral dosing to rats) with tumourigenicity but not with in-vitro mutagenic potency in Salmonella. Both of these latter two papers are appended as Annex 3 and demonstrate the importance of dose selection in deriving LED values.

25. What further evaluation is required to refine this outline scheme to an acceptable scheme for use by regulatory agencies. (e.g. consideration of rat data, and possibly some specific examples provided by regulatory agencies?)

Secretariat March 2007