

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD CONSUMER PRODUCTS AND THE ENVIRONMENT. (COM)

The Lowest Effective Dose (LED for *in-vivo* genotoxicity); a possible approach to mutagen potency ranking.

Introduction

1. The COM and COC have been discussing approaches to wider dissemination of their advice within the Comparative Risk Assessment Project. The COC agreed in principle to the use of the Margin of Exposure (MOE) approach as an additional tool to aid in risk communication on genotoxic carcinogens at its November 2006 meeting. There was no final agreement on the banding approach which could be used to rank and communicate risk (e.g. would a MOE of >10,000 equate to describing a low or >100,000 describe a very low risk) and further work was requested to expand the reported MOE estimates from food contaminant exposures to other exposures such as in air and water and to obtain external advice from a risk communication expert on the proposal. The COC agreed that the information used to communicate comparative risk would focus on the descriptions (i.e. low or very low) and not on the calculated MOEs. The agreed COM/COC approach of adopting ALARP (As Low AS Reasonably Practical) for genotoxic carcinogens is not affected by the use of the MOE approach, but the outcome of such calculations can help risk managers with prioritisation.

2. A key project raised by the secretariat during the COM horizon scanning discussion was whether the CRA project could be expanded to *in-vivo* mutagens which do not have carcinogenicity testing. One estimate arising from initial discussions during the consideration of approaches to REACH was that there would be more chemicals eventually identified as *in-vivo* mutagens but without appropriate carcinogenicity testing than those with appropriate carcinogenicity testing. The objective of this short discussion paper is to consider what approach could be used for *in-vivo* mutagens whilst retaining ALARP as the overriding policy. A suggested pragmatic approach based on the Lowest Effective Dose as proposed by Sanner and Dybing¹ has been developed. The key objective being a pragmatic approach which is of use to risk assessors and managers for internal use only.

Approaches to *in-vivo* mutagens

3. The COM guidance on a strategy for testing of mutagenicity, in common with all mutagenicity testing strategies has an *in-vitro* stage for identification of *in-vitro* mutagens, the activity of which if confirmed in *in-vivo* tests are then considered as *in-vivo* mutagens and potential human *in-vivo* mutagens. The COM guidance has a two tissue strategy for detection of *in-vivo* mutagens predominantly relying on the use of the bone-marrow as the first tissues and the liver (rat liver UDS) as the second tissue. The guidance allows for use of a range of other *in-vivo* tests to support the *in-vivo* bone-marrow test, for example DNA adduct investigations, the Comet assay, and use of transgenic rodent mutation tests. For many chemicals, particularly

those not subject to an existing regulatory testing scheme, the available in-vivo mutagenicity test data will comprise a mixture of in-vivo approaches and the extent of the data available will vary widely between chemicals.

4. The design of and protocols used for in-vivo genotoxicity and mutagenicity tests predominantly reflect the need to determine in-vivo hazard with the use of a limited number of doses to aid in hazard identification. The dose-response evaluation in in-vivo genotoxicity/mutagenicity tests is used as part of the hazard identification assessment rather than to estimate the nature of the dose-response relationship or to determine a NOEL. In contrast historically more use of the dose-response has often been made with regard to carcinogenicity tests. Thus the lowest dose in carcinogenicity tests is often selected to provide information for the determination of the NOAEL and possibly reference doses such as the ADI/TDI.

The LED potency estimate (Sanner & Dybing 2005¹ Annex 1)

5. As a pragmatic and easy way of relating mutagenic potency between chemicals, Sanner and Dybing proposed the use of the LED. The data presented in the publication focused on oral and inhalation in-vivo genotoxicity and mutagenicity studies selecting the study with positive results with the lowest effective dose (mg/kg bw) giving a significant response. A pragmatic case-by case decision of the LED was made for studies where the dose-response data had not been analysed statistically using the evaluations published by IARC. The data set used related initially to 42 chemicals using data from a number of approaches to estimating in-vivo genotoxicity/mutagenicity, including some approaches which the COM has previously considered inappropriate (e.g. SCEs). The authors compared the estimated LED with the T25 for carcinogenicity (chronic daily dose in mg/kg bw which will give 25% of animals tumours at a specific tissue site after correction for spontaneous incidence within the standard lifespan of that species). After exclusion of chemicals for which a genotoxic mode of carcinogenicity was unlikely a linear correlation was reported between LED for in-vivo genotoxicity after oral/inhalation exposure compared to T25. The authors reported that the median of the ratio LED/T25 was 1.05 and that the ratio for 90% of substances fell in the range 0.21-9.2 and reported that the LED was similar numerically to the T25 within a factor of 5-10.

Could the LED be used as a pragmatic basis for ranking in-vivo mutagens.

5. It is possible to derive a number of comments on the approach used by Sanner and Dybing. Thus the selection of the chemicals isn't fully explained. It would have possibly been more useful to provide a listing of all group 1 and 2A genotoxic carcinogens and whether the LED could be derived for these chemicals. The authors did undertake separate evaluations of rats and mice genotoxicity data and for a number of genotoxicity endpoints. However the validity of using rat genotoxicity data to derive the LED for example for butadiene and styrene but using mouse carcinogenicity data is questionable for the comparison of genotoxicity and carcinogenic potencies. In addition the use of transplacental genotoxicity data for some chemicals e.g. DEHP and doxylamine succinate but comparing to oral carcinogenicity data is also

questionable. The identity of the 34 chemicals subsequently used for the comparison of LED and T25 isn't reported. The authors only used one intraperitoneal study in the evaluation whereas the published data set of mutagenicity studies will contain a relatively high proportion of intraperitoneal studies, and hence it would be useful to consider how these data could be used in any potency ranking approach.

6. The authors suggested that the LED divided by a specified assessment factor may represent a virtually safe level for tolerable or a tolerable risk level. The value of 10,000 is quoted in the COC guidance for genotoxic carcinogens for deriving the minimal risk level but was based on a review of a selected number of genotoxic carcinogens relating the TD50/10,000 to estimated virtually safe dose levels using quantitative extrapolation of rodent tumour data. There is no proposed assessment factor published in the paper by Sanner and Dybing for mutagens. It might be possible in the future to use the same MOE bands that have been suggested to COC for carcinogens but there would appear to be considerable areas of work required to take forward the proposal for defining an appropriate MOE for in-vivo mutagens forward. Some comments are given in paragraph 11 below. It might be possible though to define crude in-vivo mutagenicity potency bands which would be very helpful to risk assessors and risk managers.

Proposal for a simplified approach to potency banding of in-vivo mutagens.

7. The COM is asked to consider the following proposal to develop a very simple approach to ranking in-vivo mutagens, which do not have carcinogenicity testing. Essentially the objective is to describe 2 or 3 potency bands for in-vivo mutagens which could aid risk assessors and risk managers when making decisions on priority and would also help to communicate comparative risk more widely. The approach is based on the COM guidance and uses those tests identified in the strategy (e.g. excludes SCE data) and is therefore confined to generally accepted methods of mutagen identification. It is intended to be used internally by regulatory agencies/Government departments only to aid in risk management decisions.

8. One of the problems of deriving an approach based on published information is that the limitations of the published data set may be highly relevant to the decisions made. Thus a chemical with relatively little published data might have a higher potency than the proposed LED value for that chemical. It is suggested that publication bias is most likely to be towards publishing positive mutagenicity data and for in-vivo DNA adduct studies, investigations with low levels of detection of DNA adducts. With regard to in-vivo DNA adduct studies, these would only be included for chemicals which had clear evidence of in-vitro mutagenicity (according to current COM guidance). Thus overall the published data are likely to be protective of public health, although there will be commercially in-confidence data for some chemicals which would not be available to risk assessors.

9. A flow diagram outlining a suggested approach to setting up the system is given overleaf, followed by a suggested initial draft flow diagram for operation. The operation of the LED approach would be modified depending

on the outcome of the initial evaluation to set the bands. The objective would not be to disseminate information on derived LED values but to place the chemical in a category e.g. low, medium and high risk priority categories. It is possible that risk assessors would make decisions on which category to place a chemical in based on a relatively quick evaluation of the available mutagenicity data and there would not necessarily be sufficient time available to fully evaluate the adequacy of all mutagenicity studies. The default would be to reject the identified LED value only if there were clear concerns regarding the quality of the critical study.

10. The operation of the banding system would need case-by case assessment, so that a chemical with an LED value near to one of the bands would require careful consideration. A pragmatic approach default would be to allocate to the higher potency band.

11. The approach outlined for in-vivo mutagens in this discussion paper focuses on deriving potency banding for in-vivo mutagens and doesn't attempt to derive MOE bands similar to those which are currently under consideration for carcinogens (e.g. an MOE of >100,000 for a genotoxic carcinogen might possibly be equated to a very low risk). The difficulty in deriving bands for MOE assessment is that there is a need to review exposure scenarios where there is clearly accepted low or very low risk situations and also to include exposure scenarios associated with risks assessed as requiring intervention possibly with different levels of urgency. Defining pragmatic mutagen potency bands could be undertaken using the available published mutagenicity data relatively simply.

12. The outcome of such an evaluation would be to conclude that for example chemical X the in-vivo mutagenic potency was in the Y mutagenicity potency band as described for known and probable human genotoxic carcinogens. There would still be an overriding requirement to adhere to ALARP, but the evaluation would aid risk managers in identifying priorities for action

COM consideration

13. Members are asked to consider a proposal for simple banding of in-vivo mutagenic potency for internal use by regulatory agencies and Government Departments to aid in risk management decisions. Could any other pragmatic and simple to apply approach be developed for in-vivo mutagens where there are no carcinogenicity data available?

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Approach to setting up LED mutagenicity potency system.

1. Select reference chemicals for defining the in-vivo mutagen bands. E.g. IARC group 1 and 2A.

2. Derive LED values for chemicals which fulfil COM guidance as both in-vitro and in-vivo mutagens. Use oral, inhalation, dermal, and intraperitoneal data. Present data for intraperitoneal studies separately. Present data for mice and rats separately. Reassess LED value only if there is a clear concern regarding the adequacy of the key study.

3. Evaluate range ranges of LED values derived from above. Can simple tertiles be derived?

Approach to using LED mutagenicity potency system.

1. Evaluate mutagenicity as per COM guidance. Proceed if chemical is an in-vitro and an vivo mutagen

2. Derive LED for appropriate route of exposure. If relevant information on route of exposure not available use lowest LED value.

3. Compare estimated LED with bands established for appropriate route or relevant band for lowest LED value if selected.

4. Place chemical in appropriate band (e.g. low, medium, high)