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CC/06/19

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

**Acute T25 - Possible approach to potency ranking of single exposure  
genotoxic carcinogens**

**Introduction**

1. The HPA and other government departments and agencies at times have to give advice on the carcinogenic risk following a single exposure to a genotoxic carcinogen, for example, following a chemical accident. Since there is evidence from animal studies that a single exposure to potent genotoxic carcinogens may be associated with higher cancer risk during later life stages, and because we make the prudent assumption that there is no threshold for genotoxic carcinogens, we can only give generic advice indicating that any carcinogenic risks from a single exposure is likely to be very small. It would be helpful if more information could be given on carcinogenic risks following such exposures. One way may be to grade the potency of genotoxic carcinogens following a single exposure. It may be that any risk from a single exposure to low potency compounds could be regarded as negligible.

2. The DH Toxicology Unit has therefore investigated the available animal data on single exposure to genotoxic carcinogens and the subsequent induction of tumours. A major problem is the limited amount of data in this area from which to assess potency and grade compounds. It was proposed that the nine most commonly studied chemicals in the Calabrese database could be the subject of a pilot study. It was considered that it would be useful to compare the rank order of potency of these compounds following single exposure to that obtained following conventional long term carcinogenicity bioassays. If the rankings were similar, it may be possible to make some assumptions about acute potency in those many cases where data are only available from chronic studies.

**Analysis of potency following acute and chronic exposure**

4. An analysis was carried out on potency (as measured by T25 values<sup>1</sup>) for the nine carcinogens for which acute data were available. This was compared with the data obtained using chronic dosing.

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<sup>1</sup> The T25 was described by Dybing and colleagues and is defined as the chronic dose rate in mg/kg bw/day which will give 25% of the animal's tumours at a specific tissue

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## Acute studies

5. Acute exposure studies have been published for the nine most commonly studied chemicals in the Calabrese database but the majority of these publications date back to the 1960/1970's. One potential difficulty with any comparison between acute and chronic calculated T25 might be that most of the chronic life-time bioassays have been undertaken using oral dosing whilst a range of routes including parenteral dosing would have been used in the single dose experiments. One approach to similar problems that has been used in risk assessment has been to assume all parenteral routes equal complete absorption and that oral toxicology data are adjusted for percent absorption. Other potential difficulties in comparisons between the chronic life-time studies and single exposure experiments would be the age of the animal at time of dosing. The majority of the acute studies were performed on newborn animals. Obtaining comparative data between chronic and acute studies for same species, strain and sex may also prove to be difficult.

6. A detailed summary is given in Annex 1 of the available acute, single exposure studies performed for each of the nine chemicals. Dose-response data were, however, only available for Dibenz(a,h)anthracene, *N*-Nitrosodiethylamine, *N*-Nitrosodimethylamine, Ethylnitrosourea, Methylnitrosourea and Benzo(a)pyrene. However, a number of these dose response studies could not be used to calculate the acute T25 values as no control data were included in the studies. The acute T25 values were calculated in a similar manner to the chronic T25, taking into account the spontaneous occurrence of the tumours in control animals (see below). In order to compare the chronic T25 and the acute T25 for the five chemicals, data from the same species was used. It was not possible to obtain data for the same strain of animal or for the same route of administration of the chemical. In the case of the chronic T25, the data was obtained from oral studies while the data for the acute T25 was obtained from a number of parenteral routes (as detailed in table 5). The comparative data of the calculated acute T25 values with chronic T25 for the five chemicals are outlined in table 5.

## Chronic studies

7. An analysis was performed to calculate the chronic T25 values for nine carcinogens. Using the paper of Dybing et al (1997) as a reference guide and using the same criteria as adopted in their materials and methods, T25 values were calculated. The criteria include calculating T25 preferentially from lifetime oral (feed or gavage) or inhalation studies in mammals, performed according to the guidelines in Annex V of the EU Directive 67/548/EEC. However, if the test

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site, after correction for spontaneous incidence within the standard life-time of that species.

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was not according to the guidelines, the following set of criteria were followed in order to calculate a T25 value: a) Animals on test were mammals, b) administration was begun early in life (preferable from time of weaning, but up to 100 days is acceptable for rats, mice and hamsters), c) route of administration was diet, drinking water, gavage or inhalation, d) the substance was bioavailable for systemic absorption, e) test agent was administered alone, f) exposure was chronic, with no more than 7 days between each dose, g) duration of exposure was at least one-fourth of standard study period for that species, h) duration of experiment was at least half of the standard lifespan for that species, i) research design included a control group, j) research design included at least 10 animals per group, k) pathology data were reported for the number of animals with tumours rather than total number of tumours, and l) results reported were original data.

8. To enable a conversion of feed, drinking water or air concentration of carcinogens to a dose descriptor needed for the T25 calculation, the factors given in Table 1 were used unless these data was provided by the study itself. In an experiment which was terminated before the standard lifespan, the number of tumours found will be reduced, and the dose rate  $d$  needed to give 25% of the animals tumours (after correction for spontaneous incidence) will then be greater than the true T25. For this reason, the true T25 was estimated as  $f_2d$ , where  $f = (\text{duration of experiment})/(\text{standard lifespan})$  (Peto et al., 1984). This is in accordance with experimental results (Druckrey, 1967). An experiment lasting for 18 months in rats with the standard lifespan of 24 months will then be corrected by  $(18/24)^2 \times d = 0.56 \times d$ . If animals are dosed 5 days per week, the dose giving 25% of animals tumours will be corrected by  $(5/7) \times d = 0.71 \times d$  to arrive at the true T25 value. If dosing is terminated at  $w$  weeks ( $w < \text{the standard lifespan of 104 weeks}$ ) (see Table 1) and the animals are observed until termination at 104 weeks, the dose giving tumours in 25% of the animals is corrected by  $w/104$ . If dosing is terminated at  $w_1$  and the animals observed until  $w_2$  weeks, the dose giving 25% of the animal's tumour is corrected by  $(w_1/104)(w_2/104)$ . It was assumed that a carcinogen is 100% bioavailable by the relevant route if specific data do not indicate otherwise.

<b>Table 1. Default values for dose calculation, experimental period, weights, and intake By diet, water, and inhalation</b>						
<b>Animal</b>	<b>Sex</b>	<b>Lifespan Years</b>	<b>Weight (grams)</b>	<b>Food/day (grams)</b>	<b>Water/day (mls)</b>	<b>Inhalation Vol L/hr</b>
<b>Mouse</b>	Male	2	30	3.60	5	1.8
	Female	2	25	3.25	5	1.8
<b>Rat</b>	Male	2	500	20.0	25	6.0
	Female	2	350	17.50	20	6.0
<b>Hamster</b>	Male	2	125	11.50	15	3.6

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	Female	2	110	11.50	15	3.6
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Taken from Gold et al 1984.

9. Using the same procedure as was used in the calculations of the TD50<sup>2</sup> values for the Gold and Zeiger database, if there was only one positive test on the chemical in the species, then the most potent T25 value from that test was reported. When more than one experiment was positive, in order to use all the available data, the reported T25 potency value was a harmonic mean of the most potent T25 values from each positive experiment. Results from the Gold and Zeiger database have shown that the harmonic mean is similar to the most potent TD50 value for chemicals with more than one positive test. The harmonic mean is one of several methods of calculating an average. The harmonic mean ( $H$ ) of the positive real numbers  $a_1, \dots, a_n$  is defined to be

$$H = \frac{n}{\frac{1}{a_1} + \frac{1}{a_2} + \dots + \frac{1}{a_n}}.$$

10. The lowest chronic T25 values using the approach set out by Dybing and colleagues and described above were calculated for the following chemicals, 3-Methylcholanthrene, Dibenz(a,h)anthracene, *N*-Nitrosodiethylamine, *N*-Nitrosodimethylamine, Ethylnitrosourea, Methylnitrosourea, Urethane, 7,12 Dimethylbenzoanthracene and Benzo(a)pyrene. Calabrese reports that these chemicals represent 58% of all studies in the databases. The calculated chronic T25 values are presented in Table 2 in order of potency ranking for hamster, mouse and rat. Table 2 and 3 represent the calculated T25 values for single species. The TD50 values for the nine chemicals are also tabulated in the same tables in order of potency. Figure 1 shows a log plot of all calculated T25 versus TD50 values for the nine chemicals. The log plot has a slope of 0.84 and a correlation coefficient ( $r^2$ ) of 0.86.

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<sup>2</sup> The TD50 is defined as the dose rate in mg/kg bw/day which, if administered chronically for the life-span of the species, will half the probability of remaining tumourless throughout that period. It requires complex statistics to calculate the TD50. Gold and Zeiger have published TD50 values for over 1300 chemicals and the estimations can be readily assessed.

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**Table 2. Potency Ranking using the calculated T25 and the TD50 of the nine chemicals for mixed species**

<b>CARCINOGEN</b>	<b>T25 (mg/kg/d)</b>	<b>CARCINOGEN</b>	<b>TD50 (mg/kg/d)</b>
DEN (Rat)	0.00972 *	DEN (Rat)	0.0265 *
MNU (rat)	0.051	7,12 DMBA (mouse)	0.084
7,12 DMBA (mouse)	0.114	MNU (rat)	0.0927
DMN (rat)	0.126 *	DMN (rat)	0.0959 *
DMN (mouse)	0.24 *	DMN (mouse)	0.189 *
B(a)P (rat)	0.24	3-MC (rat)	0.491 *
ENU (rat)	0.579 *	ENU (rat)	0.948 *
MNU (mouse)	1.546	B(a)P (rat)	0.956
Urethane (mouse)	1.85 *	MNU (mouse)	1.23
3-MC (rat)	2.08 *	DBA (mouse)	5.88
B(a)P (mouse)	7.03	B(a)P (mouse)	11.0
Urethane (Rat)	15.85	Urethane (mouse)	16.9 *
DBA (mouse)	15.92	Urethane (Rat)	41.3
Urethane (hamster)	75.75 *	Urethane (hamster)	65.2 *

\* = harmonic mean

**Table 3. Potency ranking of the chemicals for a single species (Mouse)**

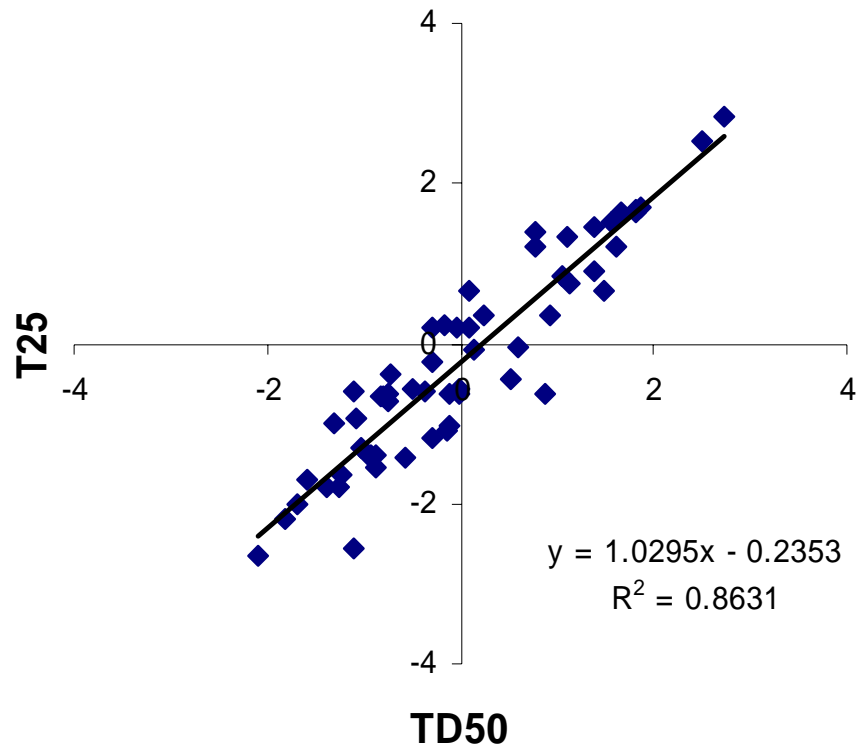
<b>CARCINOGEN</b>	<b>T25 (mg/kg/d)</b>	<b>CARCINOGEN</b>	<b>TD50 (mg/kg/d)</b>
7,12 DMBA (mouse)	0.114	7,12 DMBA (mouse)	0.084
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MNU (mouse)	1.546	MNU (mouse)	1.23
Urethane (mouse)	1.85	DBA (mouse)	5.88
B(a)P (mouse)	7.03	B(a)P (mouse)	11.0
DBA (mouse)	15.92	Urethane (mouse)	16.9

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Table 4. Potency ranking of the chemicals for a single species (Rat)

CARCINOGEN	T25 (mg/kg/d)	CARCINOGEN	TD50 (mg/kg/d)
DEN (Rat)	0.00972	DEN (Rat)	0.0265
MNU (rat)	0.051	MNU (rat)	0.0927
DMN (rat)	0.126	DMN (rat)	0.0959
B(a)P (rat)	0.24	3-MC (rat)	0.491
ENU (rat)	0.579	ENU (rat)	0.948
3-MC (rat)	2.08	B(a)P (rat)	0.956
Urethane (Rat)	15.85	Urethane (Rat)	41.3

Figure 1. Correlation of TD50 and T25 carcinogenic potency indices in a logarithmic plot.



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11. Every attempt was made to rank the nine chemicals for carcinogenicity potency using acute T25 data. It was hoped that ranking single exposure carcinogens for potency would provide valuable information for ranking priorities. However, it was not possible to acquire enough acute T25 values from single exposure/acute studies to provide any meaningful data for comparative purposes with the chronic T25 values for the nine chemicals. This was due to the lack of good quality data for the nine chemicals and due to the small number of available acute dose response studies. From the small dataset we have achieved, there does not appear to be any correlation between the chronic and acute T25 values.

**Table 5. Comparative data of the calculated Chronic T25 values with the calculated Acute T25 values**

Chemical	Chronic T25 (mg/kg/d)	Chemical	Route	Acute T25 (mg/kg)
DEN (rat)	0.00972 *	DBA (mouse)	Sc	0.56
DMN (mouse)	0.24 *	DMN (mouse)	Sc	2.56 *
MNU (mouse)	1.546	B(a)P (mouse)	Sc	3.55
B(a)P (mouse)	7.03	MNU (mouse)	lp	11.1 *
DBA (mouse)	15.92	DEN (rat)	lv	33.3

\* = harmonic mean

### Conclusions

12. It was possible to calculate the chronic T25 values for the nine chemicals, chosen from the Calabrese and Blain paper and good correlation was achieved between the TD50 values and our calculated chronic T25 values. However, although there has been a large number of single exposure studies performed for these nine chemicals over the past 50 years, the majority of these studies have involved only one dose of the chemical rather than a dose range. Also, the majority of these studies involved newborn animals. There has not been sufficient acute dose-response studies performed to enable calculation of acute T25 values for the nine chemicals and permit ranking of single exposure carcinogens for carcinogenic potency. In the case of the 5 chemicals for which there were dose-response data following acute exposure, and for which an acute T25 could therefore be calculated, there was no correlation with the ranking based on chronic toxicity.

### Questions for the committee

1. Members are asked for their comments on the above exercise.

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2. In view of the limited number of chemicals for which an acute T25 could be derived, and the results obtained, do Members consider that there is any value in taking forward the work on developing an acute T25 for carcinogenic potency? If so, members are asked what further work would be practical.
3. If Members consider that there is no value in taking forward this work, would they consider an alternative approach using the Margin of Exposure (MOE) concept (see CC/06/20)? In the event of a single exposure to a genotoxic carcinogen, a BDML<sub>10</sub> would be derived from a chronic carcinogenicity study on the chemical. A suitable MOE would then be applied to the BDML<sub>10</sub> to derive a level below which the risk of an acute exposure was considered to be negligible. This would aid officials in deciding what advice to give. If Members endorse this approach, what MOE would they consider?

### References

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