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

AGE-RELATED DIFFERENCES IN SUSCEPTIBILITY TO CARCINOGENESIS

Introduction

1. In 2003, the Committee discussed EPA draft supplemental guidance for assessing susceptibility from early-life exposure to carcinogens (CC/03/20). This was done as part of the COC consideration of the EPA draft final guidelines for carcinogen risk assessment. At last year's horizon scanning exercise, the committee saw papers by Hattis (1,2) which developed the EPA guidance further to produce risk estimates from lifetime exposure to a generic mutagenic carcinogen compared to those for adult-only exposure. Members expressed the wish to carry out a detailed review of these papers and to revisit the information examined when the committee reviewed the draft EPA guidelines. Members also commented that they would like to review the original data which was seen by the EPA.

EPA guidelines

2. The EPA has now published the final version of its "Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens" (3). The EPA identified over 50 chemicals which had been demonstrated to cause cancer following perinatal exposures in animals (without adult exposures) but only a subset had studies which compared tumour incidence after dosing with a chemical at different life stages. The studies in this subset used the following 18 chemicals:

<i>Chemicals with mutagenic* modes of action</i>	<i>Chemicals with non-mutagenic* modes of action</i>
Benzidine	Amitrole
Benzo-(a)-pyrene (B{a}P)	DDT
Dibenzanthracene (DBA)	Dieldrin
Diethylnitrosamine (DEN)	Diphenylhydantoin
Dimethylbenz(a)anthracene (DMBA)	Ethylenethiourea (ETU)
Dimethylnitrosamine (DNM)	Polybrominated biphenyls (PBBs)
Ethylnitrosourea (ENU)	
3-Methylcholanthrene (MCA)	
N-methylnitrosourea (MNU)	
Safrole	
Urethane	
Vinyl chloride	

* EPA designation

There were data from 'repeated' (juvenile or adult) exposures or 'lifetime' (combined juvenile and adult) exposures for 6 genotoxic chemicals and 6 non-genotoxic chemicals (1).

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In the other studies, exposure was acute: often only one dose was given to different study groups at different life stages, and these were often given i.p. The available studies are summarised in Table 1 ('repeated dose' and 'lifetime' studies) and Table 2 (acute studies), taken from the EPA document. From the 'repeated dose' studies on the genotoxic chemicals, the EPA calculated that there was approximately a 10-fold increased sensitivity from early life as compared to adult-only exposure. However, the susceptibility was tissue dependent: there was greater susceptibility from early-life exposures for neoplasms of the CNS, liver, mammary gland, reticular tissues, thymus, lymph and kidney and leukaemia; and less susceptibility from early-life exposures for neoplasms of the forestomach, oesophagus and harderian gland. Neoplasms of the lung showed equal or possibly greater susceptibility from early life exposures (4).

3. The EPA used the data from the 'repeated dose' studies on the genotoxic chemicals to develop adjustment factors in their quantitative estimates of lifetime cancer risk for genotoxic chemicals (4,5). (The 'lifetime' exposure studies were considered too insensitive for estimating relative juvenile:adult sensitivities). The 4 chemicals used in the 'repeated dose' studies¹ were considered to be representative of all genotoxic chemicals. The adjustment factors derived were 10-fold for exposures from birth to 2 years of age and 3-fold for exposures from 2 to 15 years of age, inclusive. For non-genotoxic chemicals, no general adjustment was recommended because there was considered to be insufficient information or analyses available to determine a general adjustment at present.

4. When the COC considered these data in 2003, members considered that it was difficult to draw any conclusions from the EPA comparisons between results with conventional carcinogen bioassays and studies involving early postnatal and juvenile exposure because of the variability in the study designs used for the latter studies. The committee commented that there were few pharmacokinetic data to allow a comparison to be made between the systemic doses achieved after oral exposure in the conventional assays and the parenteral exposures often used in the studies with early postnatal and juvenile exposure. Also, there was only limited consideration of mechanisms and of the target tissues. The committee agreed that there was some biological plausibility that there would be increased sensitivity in early life and the analysis provided limited data to support this, but that this was not always the case. As the COC does not use the slope of the dose response from animal bioassays to calculate human cancer risks (and the estimation of tolerable exposure levels) it was agreed that the adjustment factors proposed by the EPA were not relevant to the UK. The committee also concluded that there was no evidence that the use of conventional cancer bioassays in animals would fail to detect chemical carcinogens.

5. Other problems which arise in interpreting these studies are lack of knowledge about the exact dose received *in utero* following maternal exposure, or by the nursing pups when the chemical is received via the mother's milk. Many of the studies were old and did not use a standard design. Moreover, time to tumour information was not recorded.

Hattis et al (2004) (1; Appendix 1)

¹ The relevant chemicals were: benzidine, 3-methylcholanthrene, safrole and vinyl chloride.

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6. Hattis et al (2004) presents a new analysis of the studies reviewed by the EPA, supplemented by a few additional data. The data on DDT and dieldrin were deleted from the analysis because of the complexity of the dosing protocols used in the relevant studies. Where the doses in the original studies had been expressed as concentrations in the diet, air or water, they were entered into the analysis as such, but where they were expressed as micrograms per kg bw or similar (when administered by i.p. or other injections) they were transformed into micrograms per (kg bw)^{0.75}, using standard values for body weights.

7. The authors also transformed the raw observations of tumour incidence at a site in different dose groups into the estimated number of tumour transformations per animal at that site and compared the rates of transformation in different age groups with that in adult animals (methodology given in paper). They then derived separate summary relative potency estimates for the *in utero* (8 days beginning on GD12), birth-weaning and weaning-60 days periods.

8. Using the whole data set, the results of the analysis indicated that exposure from birth to weaning was the most effective period for inducing tumours, with increased sensitivity also following *in utero* exposure (Table 3 of Appendix 1). Using the results from the 'repeated dose' and 'lifetime studies' only (now termed 'continuous dosing'), different results were obtained for the genotoxic and non-genotoxic chemicals. For the genotoxic chemicals, the sensitivity in the *in utero*², birth-weaning and weaning-60 days period were 8.4, 24 and 3.7 times greater than with adult dosing only. For the non-genotoxic chemicals, there was no increased sensitivity in the *in utero*³ and weaning-60 days periods and only 3 times higher sensitivity in the birth-weaning period (Table 4 of Appendix 1).

9. There was some indication from the combined data sets for genotoxic carcinogens that males may show a greater excess sensitivity relative to adults than females (Table 8 of Appendix 1), and that rats may show greater sensitivity for the *in utero* and weaning-60 day life stages than mice (Table 9 of Appendix 1).

10. Using the data from acute dosing studies, Hattis *et al* (1984) then compared relative sensitivities for 4 genotoxic carcinogens which require metabolic activation to the genotoxic form with those of 2 direct-acting genotoxins (MNU and ENU). The only difference was for the *in utero* exposure periods. Whereas the relative sensitivity was 10-fold greater than with adult-only exposure for the direct-acting genotoxins, it was less than that with adult-only exposure for the indirectly acting genotoxins. Thus the lack of metabolising capacity in the fetus appears to offer protection from genotoxic carcinogens which require metabolic activation, as might be expected.

11. To assess whether exposure via the mother's milk indicated similar sensitivity to that from direct exposure to pre-weaning animals, the authors compared data from studies in which genotoxic carcinogens were given in the diet to nursing dams and those in which chemicals were given direct to the pups. Table 12 of Appendix 1 indicates that the

² Although Table 4 of Appendix 1 states that this analysis was based on data for 5 compounds, there only appear to be two studies entailing *in utero* exposure of two 'continuous exposure' genotoxic chemicals: benzidine and safrole.

³ *In utero* data on amitrole only

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lactational exposures resulted in higher sensitivity in the preweaning period relative to adult-only exposure than direct exposures. The authors suggest that one possible explanation is that some of the bolus doses given in the direct administration studies may have partially saturated metabolic activation pathways, leading to a less effective dose of DNA-reactive metabolites per unit exposure than when such chemicals are administered more slowly via milk.

Hattis et al (2005) (2; Appendix 2)

12. This paper develops the analysis in the earlier paper to draw implications for assessing human risks for full lifetime exposures. The analysis takes into account 3 types of uncertainties entailed in using the rodent data to make risk estimates in future. The most important of these is considered to be uncertainty in mapping of rodent life stages to human ages/exposure periods and this is discussed further below. The paper concludes that the estimated population arithmetic mean risk from lifetime exposures at a constant mg/kg bw dose to a generic genotoxic carcinogen was approximately 2.8-fold larger than expected from adult-only exposure (95% CI 1.5-6). For the EPA's 0-2 and 2-15 year age groups, they found mean expected risk increments of 13.7- and 4.7-fold relative to mean adult exposure risks, respectively. The bottom line conclusion from the paper is that the results imply that probably most of the total lifetime risk of cancers from continuous mg/kg bw/day exposures to genotoxic carcinogens arises from exposures that are received before adulthood.

13. To produce estimates of comparable life stages in mice, rats and humans, the authors decided to use weight-related estimates of relative age in the context of the times of onset of sexual maturity in the different species. The times of onset of sexual maturity in mice and rats which were used are given in Table 3:

Table 3 : Species differences in times of beginning sexual maturity (from Kilborn, 2002 (6))

Species (time unit)	Male	Female
Mouse (months)	1.5	1.0
Rat (months)	1.8-2.1	1.8-2.1
Human (years)	11.5	10.5

14 The paper calculated the fraction of their body weights at sexual maturity which rats and mice achieve at the limits of the three exposure periods used in the above analysis. Then the ages at which average humans of each sex achieve the same fractions of the body weights at sexual maturity were calculated. The results are given in Tables 4 and 5 below, using mice and rats respectively, for comparison.

Table 4: Mice: inferences of corresponding human ages from weight-based comparisons relative to the times of sexual maturity (from Hattis et al, 2005)

Dosing period	Corresponding human ages from data on	
	Male mice	Female mice

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In utero (GD12 – birth)	GD93 - GD245	GD112 –14 days
Birth – weaning	GD245 – 3.16 yrs	14 days – 7.4 yrs
Weaning – 60 days	3.16 yrs – 12.8 yrs	7.4 yrs – 15.1 yrs

GD: gestational days; days: days of age; yrs: years of age

Table 5: Rats: inferences of corresponding human ages from weight-based comparisons relative to the times of sexual maturity (from Hattis et al, 2005)

Dosing period	Corresponding human ages from data on	
	Male rats	Female rats
In utero (GD12 – birth)	GD66 – GD196	GD66 –210 days
Birth – weaning	GD196 – 0.44 yrs	14 days – 0.90 yrs
Weaning – 60 days	0.44 yrs – 11.7 yrs	0.90 yrs – 10.6 yrs

GD: gestational days; days: days of age; yrs: years of age

15. The authors note that the human ages corresponding to rodent weaning (21 days of age in rats and mice) show a large variation between projections from mouse data compared to those from rat data and, within each rodent species, between males and females. This adds a further uncertainty into any attempt to derive risk estimates for carcinogenic potency at different life stages. (However, interestingly, the rat data – but not the mouse data – produces results which corresponds well to the time of onset of sexual maturity in humans).

Questions for the committee

16. The committee is asked to address the following questions:

- i) What are Members' views on the analysis by Hattis et al (2004)? Does the committee agree with the paper's conclusions about the relative sensitivities to carcinogens in the *in utero* (GD12 – birth), birth - weaning, and weaning - 60 day periods?
- ii) Does the committee agree that the results of the analysis imply that probably most of the total lifetime risk of cancers from continuous mg/kg bw/day exposures to genotoxic carcinogens arises from exposures that are received before adulthood?
- iii) What are Members' views on the method used in Hattis et al (2005) to produce estimates of comparable life stages in mice, rats and humans?
- iv) At the horizon scanning discussion, Members expressed a wish to review the original data which was seen by the EPA. However, the risk assessment advice for genotoxic chemicals in the UK is to keep exposures as low as practicably achievable, and a review of relative adult and juvenile sensitivities for genotoxic chemicals would not affect this advice. Would Members like to review the data on non-genotoxic chemicals in more detail?

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Secretariat
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