

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

**THE MINIMUM DURATION OF CARCINOGENICITY STUDIES IN RATS**

**Introduction**

1. The life-span of some strains of rat used in carcinogenicity studies has declined, and has led to compromised statistical credibility of negative results at termination of such studies at 24 months. The 24 months study termination point is specified in international guidelines for rat carcinogenicity studies (e.g. OECD 453 (1981), 87/302/EEC, EPA 83-1). No such life-span decline has been apparent in mice. The reasons for this decline in life-span have been considered previously by the Committee on Carcinogenicity, (see statement on 'Longevity in carcinogenicity studies in rats', 2000, appendix 1). Terminating rat carcinogenicity studies at 18 months would be advantageous as this appears to be a time when survival is often still adequate to allow for a satisfactory statistical analysis of tumour incidence. Another advantage would be that of reduced time and cost in generating such a study. However reducing the duration to 18 months might not be a solution in the long run as life-spans may continue to decline and studies may need to be terminated earlier and earlier. This paper considers the effect of early termination on the capacity of rat carcinogenicity studies to detect carcinogenic potential. If this capacity was unaffected, one option for circumventing the problem of declining rat life-spans in carcinogenicity studies would be to reduce the duration of the study. Other options might include refinement in the strains of rats used in studies, or refinement of husbandry to achieve satisfactory survival during studies e.g. the introduction of a new specified diet ('NTP 2000') has significantly improved survival of Fischer 344 rats used in chronic studies (*Rao et al*, 2001). There would need to be convincing evidence of the acceptability of sub 24 month rat carcinogenicity study duration if a recommendation were to be made to OECD to change guidelines.
2. Several published papers have considered the effects of reducing rat carcinogenicity study duration from 24 to 18 months. The two papers considered in detail here are Davies *et al* (2000) and Kodell *et al* (2000), and both reach contrary conclusions. Davies *et al* examined whether the 'power' of the rat carcinogenicity study to detect potential carcinogens would be lost if the study were shortened to 18 months and concluded it would not. Kodell *et al* conducted a review of studies to examine whether statistical power of assays might be lost if the study were shortened to 18 or 21 months vs. 24 months. Kodell *et al* concluded statistical power would be lost if studies were terminated at 18 or 21 months.

**Davies et al, 2000**

3. Davies *et al* studied IARC chemical Monographs (Vols 1-70) to determine the time of onset to 'treatment-related' tumorigenicity in long-term rodent studies for chemicals classified by the IARC as showing evidence of carcinogenicity in animals. Davies *et al* did not explain how they determined the first

treatment related tumour in a study, or how they distinguished it as being in excess of the control incidence (see also paragraph 6). The chemicals were categorised as producing tumours at <12m, 12-18m, or >18m. See appendix 2 for a list of chemicals examined by Davies *et al.* The analysis excluded studies on metals and their salts, studies on particulates, studies by parental routes of administration that resulted in tumours only at the site of exposure, and studies that did not approximate to the current standard long term rodent carcinogenicity bioassay (OECD 453 (1981)), e.g. transplacental or multigeneration studies, initiator-promoter studies, lung tumour assays in 'Strain A' mice and studies in new born animals. Davies *et al* considered that from a total of 210 chemicals, overall, evidence of treatment related tumorigenicity was first apparent within 12 months for 66% of the chemicals and that studies longer than 18 months were necessary for 7%. All IARC Group 1 chemicals were detected in animals within 18 months and most within 12 months. Most of the tumour types that required more than 18 months for detection were considered by Davies *et al* to be of "dubious" relevance to human risk assessment (see appendix 2(vii) ). On this basis Davies *et al* concluded that termination of rodent carcinogenicity studies at 18 months or earlier was justified, and would greatly reduce the complications that arise in interpreting findings in aged animals. Davies *et al* stated that aged animals often have defective hepatic or renal function and would also markedly reduce the time required for histopathological examination of dozens of tissues taken from the approximately 500 animals routinely employed in these studies. Also in favour of their conclusion, Davies *et al* argued that it is possible that an earlier onset of the incidence of a common spontaneous tumour type could be detected at 18 months and missed at 24 months. Data from 18 month studies would be cleaner and easier to interpret, and would not be clouded or possibly obscured by the age related pathology that is present in most rodents at 24 months.

### **Kodell *et al*, 2000**

4. Data from drug studies in rats were used to formulate biologically based dose-response models of carcinogenesis based on the 2-stage clonal expansion model. These dose response models, which were chosen to represent 6 variations of the initiation-promotion-completion cancer model were employed to generate a large number of representative bioassay data sets using Monte Carlo simulations. The six variations of the model were based on data:

<b><u>Model Variation</u></b>	<b><u>Data on which model variation was based</u></b>
initiator only	anonymous drug 1 and pancreas adenoma in females.
completer only	anonymous drug 1 and mammary adenocarcinoma in females
initiator+completer	anonymous drug 1 and mammary adenocarcinoma in males
initiator+promoter	anonymous drug 2 and pancreas acinar cell carcinoma in males
promoter+completer	anonymous drug 3 and thyroid follicular cell adenoma in males
promoter only	selenium sulphide and liver hepatocellular carcinoma in females

5. For a variety of tumour dose-response trends, tumour lethality and competing risk-survival rates, the power of age-adjusted statistical tests to assess the significance of carcinogenic potential was evaluated at 18 and 21 months and

compared to the power at the normal 24 month termination time. The statistical analysis of each generated experimental data set was carried out using the standard IARC context of observation (cause-of-death) approach as outlined by Peto et al (1980). Kodell *et als* results showed that termination at 18 months would reduce statistical power to an unacceptable level for all 6 variations of the 2-stage clonal expansion model, with the pure-completer models being most adversely affected. There were too many combinations of tumour-onset, tumour lethality and competing risk-survival models for which the relative efficiency to 24 months is reduced too greatly to warrant termination as early as 18 months. For termination at 21 months, the results showed that unless pure promotion can be ruled out *a priori* as a potential carcinogenic mode of action, the loss of power is too great to warrant early stopping.

### **Discussion**

6. It is not considered that the overall outcome of the study by Davies *et al* (ie that a study duration of 18 months appeared acceptable in most cases) was a surprising outcome since the chemicals studied included a high proportion of IARC Group 1 and 2A carcinogens, which would be expected to be genotoxic and to result in relatively rapid onset of carcinogenesis. Davies *et al* did not differentiate between mechanisms of carcinogenesis. This is in contrast to Kodell *et al*, who examined six models. They also considered that the chemicals which induced tumours after 18 months were of dubious relevance to humans. However these chemicals included 2 compounds catagorised by IARC as 2A (probably human carcinogens) and these are both clearly genotoxic carcinogens namely acrylamide and p-chloro-o-toluidine; these data are relevant to humans. Also, many of the other compounds that were only detected after 18 months were non-genotoxic carcinogens. One of the justifications for carrying out a carcinogenicity bioassay in animals is to detect such compounds. According to Haseman *et al* (2000), what Davies *et al* considered to be a “treatment related tumorigenic effect” often reflected the occurrence of a single neoplasm, with most tumors occurring much later in the study. Reliance on a single tumor at an early time point as providing definitive evidence of rodent carcinogenicity could produce both false positive and negative outcomes. Haseman *et al* also concur with Kodell *et al* that many rodent carcinogens produce later-appearing tumors that would not be detected as statistically significant in a 12-18 month study. Haseman *et al* point out that such a shortened duration study would be roughly equivalent to evaluating human cancer in subjects 30-50 years of age, which would result in markedly reduced study sensitivity. Davies *et al* also argued that it is possible that an earlier onset of the incidence of a common spontaneous tumour type could be detected at 18 months and missed at 24 months. However it is considered likely that these would be detected if they resulted in the death of the animal. However it is considered that autopsy of the dead animals and an analysis of tumour incidence in decedents would pick this up.
7. Davies says there are good grounds for shortening to 18 months on the grounds that no tumours of relevance to human risk assessment would be identified. However it is considered that some significant carcinogens relevant

to humans would be missed. Kodell *et al* also considered “that stopping at 18 months would reduce statistical power to an unacceptable level for all 6 submodels examined, with the pure-cumulative models being most adversely affected. For the 21 month stopping time, the results showed that unless pure promotion can be ruled out *a priori* as a potential carcinogenic mode of action, the loss of power is too great to warrant early stopping.”

8. If reduced survival is the result of acceleration of the total ageing process, then the measured endpoints of survival and carcinogenesis might not be independent. That is, the cancer process might accelerate at the same rate as the overall ageing process. If this were so, then it would not be correct to assume the mutual independence of some parameters as in the Kodell *et al* models (time to tumour onset, time to tumour death, and time to death from a competing risk). In studies carried out at the National Centre for toxicological research, *ad libitum*-fed F344 rats developed greater numbers of spontaneous tumours than calorically restricted animals, despite the significantly shorter life-span of the *ad libitum*-fed animals (Thurman *et al*, 1994). If the same phenomenon were to occur with chemically induced tumours, then decreased survival might not reduce the power to detect carcinogens at all. However, for reliable detection of carcinogenic potential, bioassays should have good survival and relatively low-background tumour rates in order to minimise confounding of the various pathological changes resulting from exposure to chemical agents with those of ageing.

### **Draft Conclusion**

9. Detailed Monte Carlo modelling of tumorigenesis by Kodell *et al* demonstrated inadequate statistical power of studies if terminated short of 24 months, at 18 or 21 months. Davies *et al* also demonstrates significant human carcinogens may be overlooked if studies were terminated at 18 months. There is however evidence from Thurman *et al* that the cancer process might accelerate at the same rate as the overall ageing process in rats with reduced longevity, and decreased survival might not reduce the power to detect carcinogens. However, it is desirable for bioassays to have good survival and relatively low-background tumour rates in order to minimise potential confounding of the various pathological changes resulting from exposure to chemical agents with those of ageing. Thus the duration of rat carcinogenicity studies should be 24 months in line with current internationally accepted guidelines for testing.

**Secretariat  
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**References**

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## Appendix 1

## **Longevity in carcinogenicity studies in rats: analysis of a database prepared by PSD**

COC Statement - April 2000 - COC/00/S3

### **Introduction**

1. The proper conduct of carcinogenicity studies in rats is an important part of the evaluation and prediction of potential human carcinogens. Significant reductions in the number of control rats surviving to termination have been widely reported in the scientific literature. (1-5) This is a matter of concern since inadequate carcinogenicity studies could be important in decisions regarding the identification of potential human carcinogens and in particular the failure to identify such compounds. In addition there is a possibility that inadequate studies could be rejected by regulatory agencies with the consequent need for use of further animals to obtain a valid result. For a negative result from a rat carcinogenicity bioassay to be considered acceptable, survival at 24 months should be 50% or greater in all groups (see OECD, EPA and EC guidelines).

### **COC consideration of database prepared by Pesticides Safety Directorate(PSD)**

2. PSD reviewed survival in control animals from 26 carcinogenicity studies in rats which had been submitted over the period 1993-1998. These carcinogenicity tests had been undertaken between 1983 and 1995. Of these studies 18 had used Sprague-Dawley rats (from various sources), six Wistar rats and two Fischer 344 rats. Adequate survival was reported for 3/18 studies in Sprague-Dawley rats, all of the studies in Wistar rats, and one study undertaken in Fischer 344 rats. Most inadequate studies had been undertaken using Charles-River Sprague-Dawley rats. There was no evidence to support previous suggestion (6) that Virus Antibody status had an effect on survival.

### **COC consideration of FDA proposals for dietary restriction**

3. The FDA published a Points-to-Consider document in 1996.(1) It was recommended that ..."sponsors and petitioners consider variability in outcome that results from ad-libitum feeding and other factors influencing body weight such as multiple housing, feeder type, and cage type, and take appropriate steps to control these variables to improve the interpretation and regulatory utility of studies conducted in support of submissions, through dietary control and establishment of idealised body weight growth curves."

4. The COC considered some recently published information on diet and caloric restriction in rodent carcinogenicity bioassays.(5) The Committee reiterated the conclusion published its 1991 "...the most suitable nutritional regime, or the desirable body weight, have yet to be agreed because of the paucity of information. There is, therefore, no general agreement about the value of altering the dietary regime in carcinogenicity tests.The decision to do so must rest at present with the individual toxicologist".(7) The Committee considered that individual housing of animals such as that required for dietary restriction was not desirable for animal welfare reasons. Other relevant points to note were that the consequences of dietary restriction even on responses to known carcinogens, for instance in terms of their kinetics and metabolism, are insufficiently documented. In addition the appropriate duration of experiments in such longer-lived animals is also not known.

### **Conclusions**

5. The Committee reached the following conclusions;

A. Information from the database of rat carcinogenicity studies reviewed by PSD supports the view that unacceptable survival at termination (<50%) in carcinogenicity tests is predominantly confined to Charles-River Sprague-Dawley rats. Survival in long-term carcinogenicity bioassays should be compliant with current UK and EC guidelines for the

acceptability of a negative result from such studies.

B. The available information supports the view reached by the COC in its guidelines published in 1991 that dietary restriction in carcinogenicity studies should be applied with caution and is the responsibility of the toxicologist undertaking the study. This subject may be reviewed when more information is available.

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## References

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## Appendix 2

Chemicals examined by Davies *et al* (2000), as categorised by Davies *et al*.

## (i) Chemicals for which a tumorigenic effect was observed at or before 52 weeks in rats and/or mice

Acrylonitrile	2-(2-Formylhydrazine)-4(5-nitro-2-furyl)thiazole
Adriamycin	2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2)
Aflatoxin B <sub>1</sub>	Griseofulvin
2-Amino-5-(5-nitro-2-furyl)1,3,4-thiadiazole	Hexachlorobenzene
<i>o</i> -Aminoazotoluene	Hydrazine
2-Aminodipyrido (1, 2 $\alpha$ :3, 2d)-imidazole {Glu-P2}	Isoprene
2-Amino-9 <i>H</i> -pyrido[2,3 <i>b</i> ]indole {A- $\alpha$ -C}	Medroxyprogesterone acetate
<i>o</i> -Anisidine	Methylazoxymethanol
Azacytidine	4,4 -Methylenebis 2-chloroaniline
Azaserine	2-Methyl-1-nitroanthraquinone
Benzidine	<i>N</i> -Methyl- <i>N</i> -nitro- <i>N</i> -nitrosoguanidine (MNNG)
Benzyl violet	4-(Methyl-nitrosamino-1-pyridyl)-1-butanone (NNK)
Bis-(2-chloroethyl) nitrosourea (BCNU)	<i>N</i> -Methyl- <i>N</i> -nitrosourea
Bracken fern	Methylthiouracil
Carbon tetrachloride	Metronidazole
Carrageenan (degraded)	5-(Morpholinomethyl)-3-((5-nitrodurylidene)aminol-2-oxazolidinone
Chlorinated paraffins (C 12)	5-Nitroacenaphthene
C.I. Acid Red 114	2-Nitrofluorene
C.I. Direct Blue 15	<i>N</i> -[4-(5-Nitro-2-furyl)-2-thiazoyl]acetamide
Clitoral gl.	1-[5(-Nitrofurylidene)amino]-2-imidazolidine
Coal tar	Nitrogen mustard
Creosote oil	Nitrogen mustard <i>N</i> -oxide
<i>p</i> -Cresidine	2-Nitropropane
Cycasin	1-Nitropyrene
Dacarbazine	4-Nitropyrene
Daunomycin	<i>N</i> -Nitroso-di-ethanolamine
<i>N,N</i> -Diacetylbenzidine	<i>N</i> -Nitrosodimethylamine
2,4-Diaminotoluene	<i>N</i> -Nitroso-di- <i>n</i> -butylamine
7 <i>H</i> -Dibenz[ <i>c,g</i> ]carbazole	3-( <i>N</i> -Nitrosomethyl amino)propionitrile
Dibromochloropropane	<i>N</i> -Nitrosomorpholine
1, 2-Dibromoethane	Nitrosornicotine
1, 4-Dichlorobenzene	<i>N</i> -Nitrosomethylvinylamine
3, 3-Dichlorobenzidine	<i>N</i> -Nitrosornicotine (NNN)
1,2-Diethylhydrazine	<i>N</i> -Nitrosopiperidine
Di isopropyl sulfate	<i>N</i> -Nitrosopyrrolidine
<i>Trans</i> -2-(Dimethylamino)methylimino-5-[2-(5-nitro-2-furylvinyl)]1,3,4-oxadiazole	Oxazepam
1,1-Dimethylhydrazine	Panfuran S
1,2-Dimethylhydrazine	Phenacetin
1,6-Dinitropyrene	Phenobarbital
1,8-Dinitropyrene	<i>o</i> -Phenylphenol, sodium salt
2,4-Dinitrotoluene	Phenytoin
2,6-Dinitrotoluene	Polychlorinated biphenyls
Direct Black 38	Ponceau MX
Direct Blue 6	Ponceau 3R
Direct Brown 95	Potassium bromate
Ethyl methanesulfonate	Procarbazine
Estradiol 17(-esters)	Sterigmatocystin
Estrone	Streptozotocin
<i>N</i> -Ethyl- <i>N</i> -nitrosourea	Styrene oxide
	Sulfallate

Testosterone propionate  
Tetrachloroethylene  
4,4-Thiodianiline  
Thiotepa  
Thiourea  
Trichloromethine  
1,2,3-Trichloropropane  
Trypan blue  
Urethane  
Vinyl chloride  
Vinyl fluoride

**(ii) Chemicals for which a tumorigenic effect was observed at or before 52 weeks in rats and at more than 52 weeks in mice**

Acetamide  
3-Amino- 1,4-dimethyl-5*H*-pyrido[4,3*b*]indole  
(Trp-P- 1)  
2-Amino-3-methylimidazo[4,5-*f*]quinoline  
(IQ)  
Bromodichloromethane  
Chloroform  
1-Chloro-2-methylpropene  
4,4-Diaminodiphenyl ether  
1,2-Dichloroethane  
Diglycidyl resorcinol ether  
Dihydroxofrole  
1,4-Dioxane  
Furan  
Mestranol  
Nitrilotriacetic acid (tri-sodium salt)  
2-Nitroanisole  
*N*-Nitrosodiethylamine  
*N*-Nitrososarcosine  
Progesterone  
Propylthiouracil  
Safrole  
Tamoxifen  
Thioacetamide

**(iii) Chemicals for which a tumorigenic effect was observed at or before 52 weeks in mice and at more than 52 weeks in rats**

Amitrole  
Auramine  
Benzene  
Benzo[*a*]pyrene  
Benzofuran  
1,3-Butadiene  
Cyclophosphamide  
Diethylstilbestrol  
Hexachlorocyclohexane ( $\alpha$  and tech. grade)  
Polybrominated biphenyls  
2,3,7,8-TCCD  
1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane  
(DDT)

**(iv) Chemicals for which a tumorigenic effect was first observed between 53 and 80 week in rats and/or mice.**

2-Amino-3-methyl-9*H*-pyrido[2,3,*b*]indole  
{MeA- $\alpha$ -c}  
3-Amino-1-methylimidazo-5*H*-pyrido[4,3,*b*]-  
indole (Trp-P-2)  
*p*-Aminoazobenzene  
4-Aminobiphenyl  
Butylated hydroxyanisole  
Bis(chloromethyl) ether/chloromethyl methyl  
ether  
Captfol  
Chlorambucil  
*p*-Chloraniline  
Chlorinated paraffins (C23)  
1-(2-Chloroethyl)-3-cyclohexylnitrosourea  
CI. Basic Red 9  
CI. Disperse Blue  
Citrus Red No. 2  
Dantron  
2,4-Diaminoanisole  
3,3-Dichloro-4,4-diamino diphenyl ether  
Dichlorvos  
Di-(2-ethylhexyl)phthalate  
*p*-Dimethylaminoazo benzene  
2,6-Dimethylaniline  
3,3 -Dimethoxybenzidine  
1,6-Dinitropyrene  
1,8-Dinitropyrene  
Epichlorhydrin  
Ethylene oxide  
Lasiocorpine  
4,4 -Methylenebis 2-methylaniline  
Methyl methane sulfonate  
Mirex  
Monocrotaline  
Nafenopin  
Niridazole  
Nitrofen  
1-Nitropyrene  
*N*-Nitrosomethylethylamine  
*N*-Nitrosodipropylamine  
Norethynodrel  
Norethisterone  
Oil Orange SS  
Pentachlorophenol  
1,3-Propanesultone  
Propylene oxide  
Saccharin  
Tetrachloroethylene  
Toxaphene  
Vinyl bromide  
Vinylcyclohexene epoxide

**(v) Chemicals for which a tumorigenic effect was observed between 53 and 80 weeks in rats and at greater than 80 weeks in mice.**

Ethylene thiourea  
Toluene di-isocyanate  
*o*-Toluidine  
2,4,6-Trichlorophenol  
Tris(2,3-dibromopropyl)phosphate

**(vi) Chemicals for which a tumorigenic effect was observed between 53 and 80 weeks in mice and at greater than 80 weeks in rats.**

Chlordecone (Kepone)  
1,3-Dichloropropene (Telone II)  
Ethinylestradiol  
Ethylene oxide  
 $\beta$ -Naphthylamine  
Trichlorethylene

**(vii) Chemicals for which a tumorigenic effect was observed only at times longer than 80 weeks.**

Acrylamide  
Aramite  
Benzyl chloride  
Chlordane/heptachlor  
Chlorendic acid  
4-Chloro-*o*-phenylenediamine  
*p*-Chloro-*o*-toluidine  
Dichloromethane  
Ethyl acrylate  
HC Blue No. 1  
4,4 -Methylenedianiline  
Nitrobenzene  
Phenazopyridine  
Propylene oxide  
Vinylcyclohexene