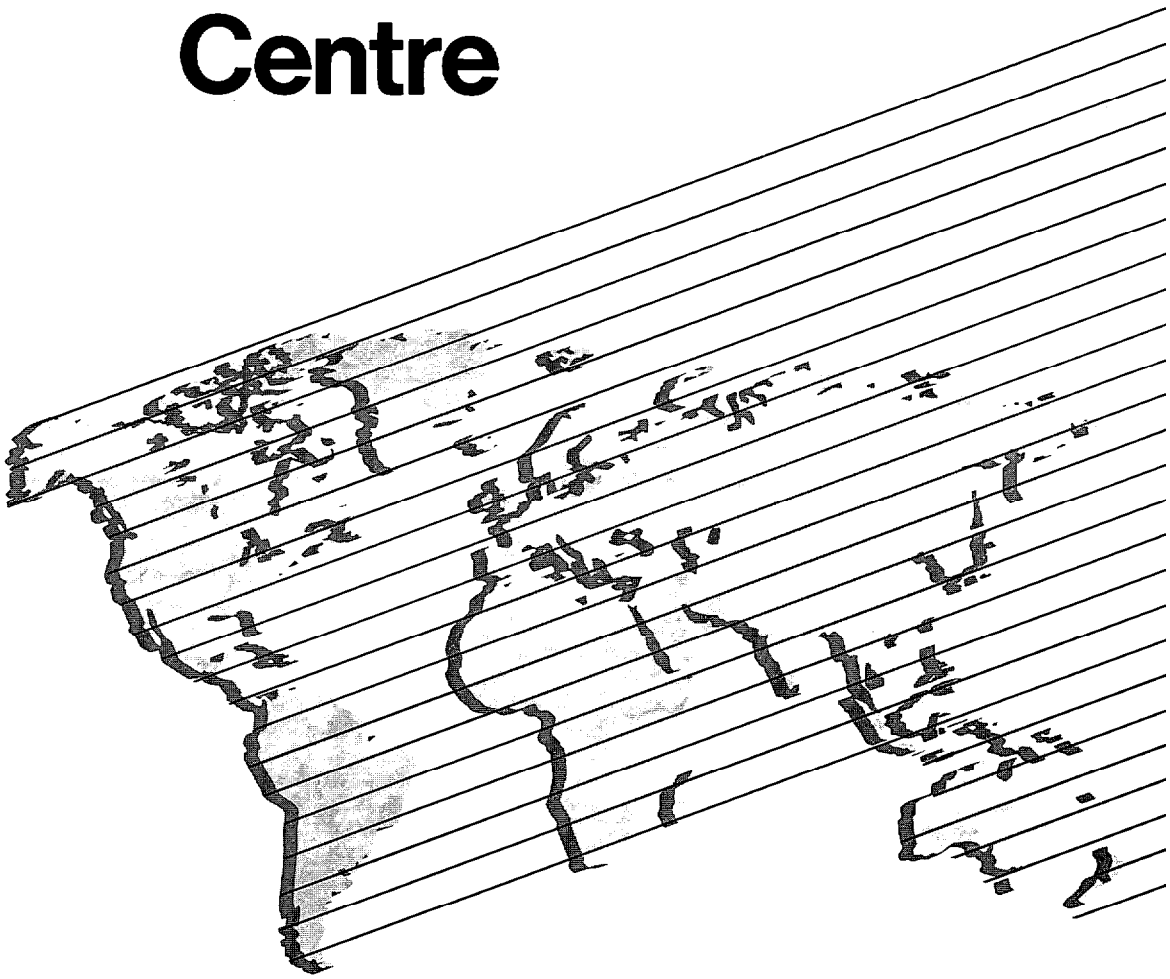
HRC Report 711/1

FLUORANTHENE

MOUSE MICRONUCLEUS TEST

Huntingdon Research Centre



DOE 12/950459

FLUORANTHENE

MOUSE MICRONUCLEUS TEST

Sponsor

Department of the Environment
Water Division
Room A322
Romney House,
43 Marsham Street,
London,
SW1P 3PY,
ENGLAND.

Testing facility

Huntingdon Research Centre Ltd.,
P.O. Box 2,
Huntingdon,
Cambridgeshire,
PE18 6ES,
ENGLAND.

Report issued 13 April 1995

CONTENTS

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health 1989.

EC Council Directive, 87/18 EEC of 18 December 1986, (No. L 15/29).

Good Laboratory Practice in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992.

United States Environmental Protection Agency, (FIFRA), Title 40 Code of Federal Regulations Part 160, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

Japan Ministry of Agriculture, Forestry and Fisheries, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

Japan Ministry of International Trade and Industry, Directive 31 March 1984 (Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85 MITI).

United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Federal Register, 22 December 1978, and subsequent amendments.

Japan Ministry of Health and Welfare, Notification No. Yakuhatsu 313 Pharmaceutical Affairs Bureau, 31 March 1982 and subsequent amendment Notification No. Yakuhatsu 870, Pharmaceutical Affairs Bureau, 5 October 1988.

R.J. Proudlock — 13 April 95

Raymond J. Proudlock, B.Sc. (Hons.), M.I.Biol.,
Study Director,
Huntingdon Research Centre Ltd.

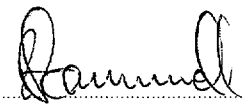
Date

QUALITY ASSURANCE STATEMENT

This report has been audited by the Huntingdon Research Centre Quality Assurance Department. The methods, practices and procedures reported herein are an accurate description of those employed at HRC during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at HRC.

Certain studies such as that described in this report, are conducted at HRC in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study described in this report was in progress, 'process-based' inspections were made by the Quality Assurance Department of critical procedures relevant to this study type. The findings of these inspections were reported promptly to the Study Director and to HRC Management.

Date(s) of inspection	3 - 28 October 1994
Date(s) of reporting inspection findings to the Study Director and HRC Management	31 October 1994
Date of reporting audit findings to the Study Director and HRC Management	14 March 1995



12-4-95

Rod Scammell,
Audit Team Supervisor,
Department of Quality Assurance,
Huntingdon Research Centre Ltd.

Date

RESPONSIBLE PERSONNEL

Raymond J. Proudlock, B.Sc. (Hons.), M.I.Biol.,
Study Director,
Department of Mutagenesis and Cell Biology.

Joanne Statham, B.Sc. (Hons.),
Assistant Scientific Officer,
Department of Mutagenesis and Cell Biology.

Wayne R. Howard,
Technician,
Department of Mutagenesis and Cell Biology.

SUMMARY

This study was designed to assess the potential induction of micronuclei by Fluoranthene in bone marrow cells of mice. Mice were treated with a single acute oral administration of the test substance by intragastric gavage at a dosage of 500, 1000 and 2000 mg/kg. A preliminary toxicity test had previously shown 2000 mg/kg, which is the standard limit dose for the micronucleus test, to be tolerated.

Negative and positive control groups were dosed in an identical manner, orally by intragastric gavage. The negative control group received the vehicle, aqueous 1% methylcellulose. The positive control group was treated with mitomycin C at 12 mg/kg bodyweight.

Bone marrow smears were obtained from five male and five female animals in the negative control and test substance groups at 2 sampling times; these being 24 or 48 hours after dosing. Bone marrow smears were obtained from the positive control group 24 hours after dosing. One smear from each animal was examined for the presence of micronuclei in 2000 polychromatic erythrocytes. The ratio of polychromatic to normochromatic erythrocytes was assessed by examination of at least 1000 erythrocytes from each animal. A record of the incidence of micronucleated normochromatic erythrocytes was also kept.

Mice treated with Fluoranthene did not show any significant increase in the frequency of micronucleated polychromatic erythrocytes at either sampling time.

There was no significant decrease in the ratio of polychromatic to normochromatic erythrocytes after treatment of the animals with Fluoranthene.

The positive control compound, mitomycin C, produced large, highly significant increases in the frequency of micronucleated polychromatic erythrocytes together with decreases in the ratio of polychromatic to normochromatic erythrocytes.

It is concluded that Fluoranthene has not shown any evidence of causing chromosome damage in this *in vivo* test.

INTRODUCTION

Fluoranthene is one of the six polycyclic aromatic hydrocarbons (PAH) which are regulated by the Water Supply (Water Quality) Regulations 1989. Although a standard for the sum of the detected concentration of these six specified PAH has been set at 0.2μg/l, there is only adequate toxicological data for quantitative assessment of benzo(3,4) pyrene, which only constitutes a minor fraction of the total PAH found in water. Contraventions of the standards in the regulations arise mainly from the presence of Fluoranthene in some drinking water; relatively high levels of Fluoranthene are thought to result from elution of this PAH from coal tar pitch, which was historically used to line cast iron water mains.

The Department of Health Committee on Mutagenicity (CoM) and the Committee on Carcinogenicity (CoC) have reviewed Fluoranthene and concluded that due to a lack of good quality *in vivo* mutagenicity and carcinogenicity data, Fluoranthene should be regarded as a potential mutagen and carcinogen unless they acquire adequate data to suggest otherwise. Therefore, in order to consider the significance of the levels of Fluoranthene in drinking water good quality *in vivo* genotoxicity studies have to be performed.

As part of this requirement, the present study was designed to assess the potential of Fluoranthene to induce chromosome damage in an *in vivo* system following acute oral administration (Boller and Schmid 1970, Heddle *et al.* 1983, MacGregor *et al.* 1987, Mavournin *et al.* 1990).

The procedures were based on the recommendations of the EEC Annex to Directive 92/69/EEC (EEC 1992) Method B12 and take into account recommendations of the UK Environmental Mutagen Society (Richold *et al* 1990) and the most recent (draft) OECD updated guideline for the assay (OECD 1994).

In mitotic cells in which chromosomal damage has been caused by the test substance or its metabolites, acentric fragments of the chromosomes do not separate at the anaphase stage of cell division. After telophase these fragments may not be included in the nuclei of the daughter cells and hence will form single or multiple micronuclei (Howell-Jolly bodies) in the cytoplasm of these cells. Micronuclei are seen in a wide variety of cells, but erythrocytes are chosen for examination since micronuclei are easily detected in this cell type.

A few hours after the last mitosis is completed, erythroblasts expel their nucleus. Young erythrocytes, less than 24 hours old, stain blue with Giemsa due to the presence of ribonucleic acid which gradually disappears so that more mature erythrocytes stain pink. The young blue-staining cells are known as polychromatic erythrocytes, and micronuclei are readily detected in this cell type. If scoring is restricted to these cells, virtually all the chromosome damage detected will have been caused during the recent exposure to the test substance.

Substances which interfere with the mitotic spindle apparatus will cause non-disjunction (unequal separation of the chromosomes at anaphase resulting in aneuploidy) or lagging chromosomes at anaphase which may not be incorporated into the daughter nuclei. These lagging chromosomes are not excluded from the erythroblast at the same time as the nucleus and hence also give rise to micronuclei.

Normochromatic erythrocytes may also be examined for the presence of micronuclei. No substantial increases in the incidence of micronuclei in normochromatic erythrocytes would usually be expected at the 24 hour sampling time after treatment with a chromosome-damaging agent; any micronucleus-like artifacts (which could otherwise possibly give a false positive result) are therefore readily distinguishable in this cell type (Schmid 1976).

Any toxic effects of the test substance on the immature nucleated cells may lead either to a reduction in cell division or to cell death. These effects in turn lead to a reduction in cell numbers and to compensate for this, peripheral blood is shunted into the bone marrow (von Ledebur and Schmid 1973). If the ratio of the polychromatic to the normochromatic erythrocytes is scored and found to be significantly less than the control value, this is taken as being indicative of bone marrow toxicity.

The protocol was approved by the Study Director and HRC Management on 17 November 1994 and by the Sponsor on 24 November 1994.

The study was performed between 7 February and 6 March 1995.

TEST SUBSTANCE

Identity:	Fluoranthene
Alternative chemical name:	1,2-(1,8-Naphthylene)benzene
CAS registry number:	[206-44-0]
Supplier:	Aldrich Chemical Co., UK.
Lot/batch number:	3357569
Expiry:	9 December 1996
Purity:	98% (GC)
Appearance:	Yellow crystalline powder
Storage conditions:	Room temperature
Date received:	9 December 1994

The above information with regard to physical characterisation of the test substance was supplied by Aldrich Chemical Co., UK.

EXPERIMENTAL PROCEDURE

ANIMALS

All animals in this study were Specific Pathogen Free CD-1 outbred mice of Swiss origin weighing between 22 and 24 grams and approximately 35 days old on despatch. The animals were obtained from Charles River U.K. Limited, Margate, Kent, England.

On arrival the weight of the animals was checked and found to be acceptable. The animals were randomly assigned to groups and tail marked. Each group was kept, with the sexes separated, in plastic disposable cages and maintained in a controlled environment with approximately 20 changes of air per hour and the thermostat set at 22°C; during the experimental and acclimatisation period, relative humidity and temperature were monitored continuously and were found to range between 48 - 52 % and 20 - 22°C respectively. The room was illuminated by artificial light for 12 hours per day. All animals were allowed free access to pelleted Biosure LAD 1 rodent diet and tap water. They were acclimatised for approximately four days, examined daily and weighed prior to dosing. Food and tap water are routinely analysed for quality at source; dietary contaminants are not suspected of having any significant effect on parameters measured in this test in this laboratory at any time over the last ten years.

TEST SUBSTANCE FORMULATION

Suspensions of Fluoranthene were prepared in aqueous 1% methylcellulose (obtained from Courtaulds, batch number T32398) on the morning of the test at the concentrations shown overleaf.

Stability and homogeneity of the test substance and of the test substance in the vehicle were not determined in this test. Chemical analysis of dosing formulations for achieved concentration was not performed.

POSITIVE CONTROL COMPOUND

Mitomycin C, obtained from BDH Limited, batch number A69321, was used as the positive control compound. It was prepared as a solution in 0.9% saline at a concentration of 0.6 mg/ml just prior to administration.

TREATMENT PROCEDURE

All animals in all groups were dosed orally by intragastric gavage with the standard volume of 20 ml/kg bodyweight. The animals were deprived of diet overnight prior to and for two hours after oral dosing.

DATES OF DOSING

Preliminary toxicity test Phase I: 7 February 1995

Micronucleus test: 21 February 1995

PRELIMINARY TOXICITY TEST

The purpose of this test was to determine a suitable dose levels for use in the main micronucleus test. This part of the study was carried out in just one phase. Eight male and eight female mice were used in this experiment. The experimental design is shown below:

Experimental design

Group	Treatment	Concentration (mg/ml)	Dose (mg/kg)	Number of mice ♂	Number of mice ♀
1	Fluoranthene	12.5	250	2	2
2		25	500	2	2
3		50	1000	2	2
4		100	2000	2	2

Following dosing, the animals were observed regularly during the working day for a period of 48 hours and any mortalities or clinical signs of reaction during the experiment were recorded. At the end of this observation period surviving animals were killed and discarded.

No further toxicity testing was performed because of the very limited toxicity shown by the test substance in the initial test.

MICRONUCLEUS TEST

From the results obtained in the preliminary toxicity study, dose levels of 500, 1000 and 2000 mg/kg bodyweight was chosen for the micronucleus test. Forty-eight male and forty-eight female mice were used in this part of the study and the experimental design is shown overleaf.

Experimental design

Group	Treatment	Concentration (mg/ml)	Dose (mg/kg)	Number of mice ♂	Number of mice ♀
1	Vehicle control	-	-	10	10
2	Fluoranthene	25	500	10	10
		50	1000	10	10
		100	2000	10+3*	10+3*
3	Mitomycin C (positive control)	0.6	12	5	5

* Additional animals, dosed concurrently, to replace any that might die

Following dosing the animals were examined regularly and any mortalities or clinical signs of reaction were recorded. Five males and five females from the negative control and test substance groups were sacrificed 24 and 48 hours after dosing. (Additional animals not used in the preparation of bone marrow smears were killed at the 48 hour time point.) The positive control group was sacrificed 24 hours after dosing. The animals were killed by cervical dislocation and both femurs were dissected out from each animal. The femurs were cleared of tissue and the proximal epiphysis was removed from each bone. A direct bone marrow smear was made onto a slide after dilution of the marrow with a drop of foetal calf serum. One smear was made from each femur. The prepared smears were fixed in methanol (> 10 minutes). After air-drying the smears were stained for 10 minutes in 10% Giemsa (prepared by 1 : 9 dilution of Gurr's improved R66 Giemsa (BDH) with distilled water). Following rinsing in distilled water and differentiation in buffered distilled water (pH 6.8), the smears were air-dried and mounted with coverslips using DPX (Proudlock and Allen 1985).

The stained smears were examined (under code) by light microscopy to determine the incidence of micronucleated cells per 2000 polychromatic erythrocytes per animal.

Micronuclei are identified by the following criteria:

(i) Large enough to discern morphological characteristics
(ii) Should possess a generally rounded shape with a clearly defined outline
(iii) Should be deeply stained and similar in colour to the nuclei of other cells - not black
(iv) Should lie in the same focal plane as the cell
(v) Lack internal structure *i.e.* they are pyknotic
(vi) There should be no micronucleus-like debris in the area surrounding the cell

The ratio of polychromatic to normochromatic erythrocytes for each animal was assessed by examination of at least 1000 erythrocytes. A record of the number of micronucleated normochromatic erythrocytes observed during assessment of this ratio was also kept as recommended by Schmid (Schmid 1976).

ASSESSMENT OF RESULTS BY STATISTICAL ANALYSIS

Non-parametric statistical methods, based on rank are chosen for analysis of results because:

(a) They are suited to analysis of data consisting of discrete/integer values such as the incidence of micronucleated polychromatic erythrocytes.

(b) The methods make few assumptions about the underlying distribution of data and therefore the values do not require transformation to fit a theoretical distribution (where data can be approximately fitted to a normal distribution, the results of non-parametric analysis and classical analysis of variance are very similar).

(c) 'Outliers' are frequently found in the polychromatic erythrocyte to normochromatic erythrocyte ratios for both control and treated animals; non-parametric analysis does not give such values an undue weighting.

Unless there is a substantial difference in response between sexes (which is rare) results for the two sexes are combined to facilitate interpretation and maximise the power of statistical analysis. For a comparison of an individual treated group with a concurrent control group, Wilcoxon's sum of ranks test is used (Hollander and Wolfe 1973, Langley 1979). An adaptation of this method (Kruskal-Wallis') is used for multiple inter-group comparisons. Jonckheere's test is used to analyse for significance of dose-related trends.

EVALUATION CRITERIA

A positive response is normally indicated by a substantial, statistically significant increase ($P < 0.01$) in the incidence of micronucleated polychromatic erythrocytes compared to the incidence for the concurrent vehicle control group for at least one of the sampling times; individual and/or group mean values should exceed the laboratory historical control range. A negative result is indicated where individual and group mean incidences of micronucleated polychromatic erythrocytes for animals treated with the test substance are not significantly greater than incidences for the concurrent control group and where these values fall within the historical control range. An equivocal response is obtained when the results cannot be adequately classified using the criteria for a positive or negative response.

Bone marrow cell toxicity (or depression) is normally indicated by a substantial, statistically significant decrease ($P < 0.01$) in the ratio of polychromatic to normochromatic erythrocytes. This decrease would normally be evident at the 48 hour sampling point, a decrease at the 24 hour time point is not necessarily expected because of the relatively long transition time of erythroid cells [late normoblast → polychromatic erythrocyte (approximately 6 hours) → normochromatic erythrocyte (approximately 30 hours)]. A very large decrease in this ratio would be indicative of a cytotoxic effect.

DATA STORAGE

All specimens, raw data and study related documents generated during the course of the study at HRC, together with a copy of the final report are lodged in the Huntingdon Research Centre Archive.

Such specimens and records will be retained for a minimum period of five years from the date of issue of the final report. At the end of the five year retention period the Sponsor will be contacted and advice sought on future requirements. Under no circumstances will any item be discarded without the Sponsor's knowledge.

RESULTS

No mortalities occurred in any group in any part of the study.

PRELIMINARY TOXICITY TEST

The details of any toxic reactions observed are given in Appendix 1.

As only minor signs of toxicity were obtained during this preliminary test, dose levels of 500, 1000 and 2000 mg/kg bodyweight were chosen for the main micronucleus test. The standard limit dose specified by EEC and draft OECD guidelines is 2000 mg/kg and we consider it to be an appropriate maximum for use in this study.

MICRONUCLEUS TEST

The results of the micronucleus test on Fluoranthene at the 24 and 48 hour sampling times are presented in Tables 2 and 3 respectively. Table 1 gives a summary of the results and the results of statistical analysis. Appendix 3 summarises the vehicle control micronucleated polychromatic erythrocyte counts obtained in previous, unrelated experiments.

Clinical signs and mortalities

No mortalities were obtained in the micronucleus test.

Clinical signs for animals treated with Fluoranthene are detailed in Appendix 2. No adverse clinical signs were obtained for the vehicle control or positive control treated animals over the duration of the test.

Micronucleated polychromatic erythrocyte counts (mnp)

Fluoranthene did not cause any statistically significant increases in the number of micronucleated polychromatic erythrocytes at a either sampling time [$P > 0.01$ using Kruskal wallis' and Jonckheere's tests].

Mitomycin C caused large, highly significant increases ($P < 0.001$) in the frequency of micronucleated polychromatic erythrocytes.

Micronucleated normochromatic erythrocytes (mnn)

Fluoranthene did not cause any substantial increases in the incidence of micronucleated normochromatic erythrocytes at either sampling time.

Ratio of polychromatic to normochromatic erythrocytes (p/n)

Fluoranthene failed to cause any significant decreases in the ratio of polychromatic to normochromatic erythrocytes [$P > 0.01$ using Kruskal-Wallis' and Jonckheere's tests]. Mitomycin C caused statistically significant decreases in the ratio [$P < 0.01$ using Wilcoxon's sum of ranks test].

CONCLUSION

Since the test substance did not cause any substantial increase in the incidence of micronucleated polychromatic erythrocytes or any substantial decrease in the p/n ratio, it is concluded that Fluoranthene did not show any evidence of causing chromosome damage or bone marrow cell toxicity when administered orally in this *in vivo* test procedure.

REFERENCES

BOLLER, K. and SCHMID, W. (1970) Chemical mutagenesis in mammals. The bone marrow of the Chinese hamster as an *in vivo* test system. Haematological findings after treatment with Trenimon (translation). *Humangenetik*, **11**, 34.

EEC (29 December 1992) *Official Journal of the European Communities* No. L383A. Part B: Methods for determination of toxicity. B12: Mutagenicity, (Micronucleus test), p.154.

HEDDLE, J.A., HITE, M., KIRKHART, B., MAVOURNIN, K., MacGREGOR, J.T., NEWELL, G.W. and SALAMONE, M.R. (1983) The induction of micronuclei as a measure of genotoxicity. A report of the US Environmental Protection Agency Gene-Tox Program. *Mutation Research*, **123**, 61.

HOLLANDER, M. and WOLFE, D.A. (1973) *Non-parametric Statistical Methods*. J. Wiley and Sons, New York & London.

LANGLEY, R. (1979) *Practical Statistics*, 2nd edition. Pan Books, London and Sydney.

MacGREGOR, J.T., HEDDLE, J.A., HITE, M., MARGOLIN, B.H., RAMEL, C., SALAMONE, M.F., TICE, R.R. and WILD, D. (1987) Guidelines for the conduct of micronucleus assays in mammalian bone marrow erythrocytes. *Mutation Research*, **189**, 103.

MATTER, B. and SCHMID, W. (1971) Trenimon-induced chromosome damage in bone marrow cells of six mammalian species, evaluated by the micronucleus test. *Mutation Research*, **12**, 417.

MAVOURNIN, K.H., BLAKEY, D.H., CIMINO, M.C., SALAMONE, M.F. and HEDDLE, J.A. (1990) The *in vivo* micronucleus assay in mammalian bone marrow and peripheral blood. A report of the US Environmental Protection Agency Gene-Tox Program. *Mutation Research*, **239**, 29.

OECD (1983) Guideline for Testing of Chemicals No. 474. Genetic Toxicology : Micronucleus Test.

OECD (May 1994) Proposal for updating guideline 474. Mammalian erythrocyte micronucleus test.

PROUDLOCK, R.J. and ALLEN, J.A. (1985) Evaluation of benzidine and 4,4"-diaminoterphenyl in the micronucleus test. In: Parry, J.M. and Arlett, C.F. (eds.) *Comparative Genetic Toxicology: the Second UKEMS Collaborative Study*. Macmillan Press Ltd., London.

RICHOLD, M. *et al.* (1990) *In vivo* cytogenetic assays. In: Kirkland D.J *et al.* (eds) *Basic mutagenicity tests: UKEMS recommended procedures*. Cambridge University Press, Cambridge.

SCHMID, W. (1976) The micronucleus test for cytogenetic analysis. In: HOLLANDER, A. (ed.) *Chemical Mutagens, Principles and Methods for their Detection*, Vol. 4, p.31. Published by Plenum Press, New York.

von LEDEBUR, M. and SCHMID, W. (1973) The micronucleus test. Methodological aspects. *Mutation Research*, **19**, 109.

TABLE 1

Summary of results and statistical analysis

Sampling time	Treatment	Dose (mg/kg)	Ratio p/n (mean)†‡	Incidence mnp (mean)†	Incidence mnn (total)
24 Hour	Vehicle control	-	0.664	1.4	0.3
	Fluoranthene	500	0.715 ns	1.1 ns	1.2
		1000	0.595 ns	1.6 ns	0.4
		2000	0.783 ns	1.2 ns	1.0
	Mitomycin C	12	0.330 *	56.4 **	1.7
48 Hour	Vehicle control	-	0.770	0.7	0.2
	Fluoranthene	500	0.737 ns	1.4 ns	0.5
		1000	0.800 ns	1.1 ns	1.0
		2000	0.795 ns	1.4 ns	0.8

p/n Ratio of polychromatic to normochromatic erythrocytes
mnp Number of micronucleated cells observed per 2000 polychromatic erythrocytes
mnn Number of micronucleated cells observed per 1000 normochromatic erythrocytes
‡ Any small apparent errors of ± 0.001 are due to rounding of individual values for presentation in tables

† Results of statistical analysis using Kruskal-Wallis', Jonckheere's and Wilcoxon's tests as appropriate:

ns	$P > 0.01$	one-sided probabilities
*	$P < 0.01$	
**	$P < 0.001$	

TABLE 2

Results for individual animals - 24 hour sampling time

Treatment	Dose (mg/kg)	Animal number	Ratio p/n	mnp	n	mnn
Vehicle control	-	201♂	1.070	1	544	0
		202♂	0.653	2	605	0
		203♂	1.016	1	499	0
		204♂	0.818	2	556	0
		205♂	0.788	0	581	0
		206♀	0.349	3	764	0
		207♀	0.688	1	698	0
		208♀	0.479	1	753	1
		209♀	0.350	3	858	0
		210♀	0.432	0	767	1
	500	211♂	0.684	0	607	1
		212♂	0.617	1	652	1
		213♂	0.918	1	522	0
		214♂	0.245	1	879	0
		215♂	0.857	1	573	2
		216♀	0.642	1	715	2
		217♀	0.433	0	927	1
		218♀	1.061	2	492	1
		219♀	0.609	3	683	0
		220♀	1.085	1	492	0
Fluoranthene	1000	221♂	0.510	3	773	0
		222♂	0.623	2	718	0
		223♂	0.614	3	625	0
		224♂	0.695	1	734	0
		225♂	0.401	1	724	2
		226♀	0.340	1	777	1
		227♀	0.523	1	694	0
		228♀	0.958	2	570	0
		229♀	0.632	1	712	0
		230♀	0.652	1	681	0
	2000	231♂	0.683	2	596	0
		232♂	0.664	1	608	0
		233♂	0.989	2	554	1
		234♂	0.653	2	623	1
		235♂	0.976	1	545	1
		236♀	0.559	0	771	0
		237♀	0.960	1	568	1
		238♀	0.730	1	586	0
		239♀	0.866	2	599	1
		240♀	0.745	0	639	1
Mitomycin C	12	241♂	0.457	52	783	4
		242♂	0.293	48	857	2
		243♂	0.342	69	879	1
		244♂	0.231	47	921	1
		245♂	0.540	65	772	0
		246♀	0.255	76	811	1
		247♀	0.162	49	864	1
		248♀	0.338	49	1032	2
		249♀	0.270	58	949	1
		250♀	0.408	51	762	2

p/n Ratio of polychromatic to normochromatic erythrocytes
mnp Number of micronucleated cells observed in 2000 polychromatic erythrocytes examined
n Total number of normochromatic erythrocytes examined for micronuclei
mnn Number of micronucleated normochromatic erythrocytes observed

TABLE 3

Results for individual animals - 48 hour sampling time

Treatment	Dose (mg/kg)	Animal number	Ratio p/n	mnp	n	mnn
Vehicle control	-	301♂	0.480	1	725	0
		302♂	1.224	0	483	1
		303♂	0.641	0	619	0
		304♂	0.813	1	654	0
		305♂	1.424	1	448	0
		306♀	0.503	0	666	0
		307♀	0.593	2	717	0
		308♀	0.979	1	564	0
		309♀	0.643	0	622	0
		310♀	0.396	1	814	0
	500	311♂	0.733	1	652	0
		312♂	0.760	0	625	0
		313♂	0.760	2	570	0
		314♂	0.609	1	677	0
		315♂	0.564	1	649	1
		316♀	0.787	2	602	1
		317♀	0.699	3	595	0
		318♀	0.896	1	608	0
		319♀	0.713	2	648	1
		320♀	0.845	1	588	0
Fluoranthene	1000	321♂	0.763	2	570	2
		322♂	0.894	1	584	1
		323♂	0.998	1	571	1
		324♂	1.162	2	469	0
		325♂	0.718	1	671	0
		326♀	0.519	2	724	2
		327♀	0.424	0	831	0
		328♀	1.054	2	497	0
		329♀	0.660	0	671	0
		330♀	0.807	0	561	0
	2000	331♂	0.716	1	663	1
		332♂	1.173	1	498	0
		333♂	0.978	1	506	1
		334♂	0.933	1	550	0
		335♂	1.324	0	476	1
		336♀	0.405	1	728	1
		337♀	0.464	2	744	0
		338♀	0.836	2	605	1
		339♀	0.604	4	672	0
		340♀	0.521	1	819	0

p/n Ratio of polychromatic to normochromatic erythrocytes
mnp Number of micronucleated cells observed in 2000 polychromatic erythrocytes examined
n Total number of normochromatic erythrocytes examined for micronuclei
mnn Number of micronucleated normochromatic erythrocytes observed

APPENDIX 1

Preliminary toxicity test

Clinical signs and mortalities

Treatment	Fluoranthene							
Dosage (mg/kg)	250		500		1000		2000	
Approx. time after dosing (hr : min)	♂	♀	♂	♀	♂	♀	♂	♀
0 : 30	P^+	P^+	P^+	P^+	P^+	P^+	P^+	P^+
1 : 00	P^+	P^+	P^+	P^+	P^+	P^+	P^+	P^+
2 : 00	P^+	P^+	P^+	P^+	P^+	P^+	P^+	P^+
4 : 00	0	P^+	P^+	P^+	P^+	P^+	P^+	P^+
6 : 00	0	P^+	P^+	P^+	P^+	P^+	P^+	P^+
7 : 00	0	0	0	P^+	P^+	P^+	P^+	P^+
23 : 00	0	0	0	0	P^+	P^+	P^+	P^+
24 : 00	0	0	0	0	P^+	0	P^+	P^+
26 : 00	0	0	0	0	0	0	P^+	P^+
31 : 00	0	0	0	0	0	0	0	0
47 : 00	0	0	0	0	0	0	0	0
48 : 00	0	0	0	0	0	0	0	0
Mortalities	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2

Degree of reaction: 0 no reaction, $^+$ slight

Type of reaction: P piloerection

Clinical signs shown refer to all animals within that dose group and sex

APPENDIX 2

Micronucleus test

Clinical signs and mortalities

Treatment	Fluoranthene					
Dose (mg/kg)	500		1000		2000	
Approx. time after dosing (hr : min)	♂	♀	♂	♀	♂	♀
1 : 00	P+	P+	P+	P+	P+	P+
2 : 00	0	0	0	0	0	0
4 : 00	0	0	0	0	0	0
6 : 00	0	0	0	0	0	0
6 : 30	0	0	0	0	0	0
23 : 00	0	0	0	0	0	0
24 : 00	0	0	0	0	0	0
30 : 30	0	0	0	0	0	0
47 : 00	0	0	0	0	0	0
48 : 00	0	0	0	0	0	0
Mortalities	0/10	0/10	0/10	0/10	0/13	0/13

N.B. No adverse clinical signs were noted for the vehicle or positive control groups throughout the experiment

Degree of reaction: 0 no reaction, + slight reaction

Type of reaction: P piloerection

Clinical signs refer to all animals within that dose group/sex

APPENDIX 3

Mouse micronucleus test

Historical control values

This summary presents a cumulative total of results for vehicle control animals used in previous, unrelated experiments during the period January 1987 to July 1994.

Cumulative results for 5110 individual animals

mnp	0	1	2	3	4	5	6	7	>7
Frequency	2653	1486	654	217	74	18	6	2	0

mnp The incidence of micronucleated cells per thousand polychromatic erythrocytes
Frequency The number of times that the result has been obtained

The individual animal mean mnp is 0.76. *

Cumulative results for 510 groups consisting of 5 male and 5 female animals

mnp	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1
Frequency	4	12	32	39	43	37	55	55	45	30	39	29
mnp	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	>2.2
Frequency	24	18	17	9	9	3	6	1	2	0	1	0

mnp The group mean incidence of micronucleated cells per thousand polychromatic erythrocytes
Frequency The number of times that the result has been obtained

The mean group mean mnp is 0.76. *

* NB These results refer to studies carried out where only 1000 polychromatic erythrocytes per animal were examined. Thus in the present study, where 2000 polychromatic erythrocytes were examined, a mean mnp value of approximately 1.5 would be expected for vehicle control or unaffected animals.

Huntingdon Research Centre Ltd
Huntingdon
Cambridgeshire PE18 6ES
England

Telephone
(01480) 890431

Fax
(01480) 890693