overview of the concawe middle distillate programme

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ABSTRACT

This report reviews the results from a three phase programme of work designed to investigate the factors influencing the skin carcinogenicity of middle distillates. In particular, it concentrates on the final phase, which consisted of a 2 year skin painting study in mice with two gas oils and a kerosine, each applied at three different concentrations.

It was concluded that materials containing significant concentrations of PACs probably produce skin tumours by a genotoxic mechanism. Undiluted straight-run gas oil and kerosine, containing low or undetectable levels of PACs may produce tumours but only when moderate to severe skin irritation is also present. Such effects are probably related to the influence of the continuous cycle of cell damage and repair prompted by chronic skin irritation.

The results are an important consideration in terms of the hazard and risk assessments for middle distillate fuels.

CONTENTS

SUMMARY

The middle distillate programme was undertaken to investigate the role of skin irritation in tumour development during long term dermal studies in mice. The programme was conducted in three phases. Phase 1 involved screening studies on 10 middle distillates. These included the determination of physico-chemical properties and PAC (polycyclic aromatic compound) concentrations, short-term *in vitro* tests mainly investigating mutagenicity and detailed histopathological examination of mouse skin following repeated dermal application of the distillates. These studies suggest that mutagenic activity is related to PAC content. In particular, relatively high mutagenic activity was evident in a cracked gas oil sample. Whereas, all of the kerosine samples which contained low levels of PACs were not mutagenic. Further it was shown that all the middle distillates were highly irritant when applied repeatedly to mouse skin.

In phase 2, five middle distillates were evaluated in dermal studies of up to 90 days duration in mice to establish dilution conditions that would enable repeated application of middle distillates without causing significant skin irritation. It was found that use of a mineral oil diluent enabled daily application of middle distillates with no more than slight irritation. Experiments with radiolabelled hydrocarbons showed that skin penetration was not reduced under these conditions. A regime was devised which enabled animals to be tested under conditions producing different degrees of skin irritation but at the same total weekly dose of middle distillate over the course of the study.

In phase 3, three middle distillates, a straight run kerosine, a straight run gas oil and a cracked gas oil, known as light cycle oil [LCO] were evaluated in a two year mouse dermal carcinogenicity study. Samples were applied undiluted and at dilutions of 50% and 28.5% by volume in mineral oil, according to the dosing regime established in phase 2. In these studies:

- undiluted straight run kerosine and straight run gas oil caused marked irritation and a number of tumours at the treatment site. Minimal or no irritation was found with the 50% and 28.5% dilutions of these distillates and no significant treatment-related tumours were found.

- the cracked gas oil (LCO) produced tumours at all three application rates. Undiluted LCO produced marked skin irritation whereas there was moderate irritation with the 50% dilution and slight irritation with the 28.5% dilution.

It is concluded that in long-term dermal studies in mice, cracked gas oils containing significant concentrations of 3 to 7 fused ring PACs probably produce skin tumours by a genotoxic mechanism. Under the same conditions, undiluted straight-run gas oils and kerosines containing undetectable or low concentrations of PACs eventually produce tumours, but only when moderate to severe skin irritation is also present. These tumours are probably the consequence of a continuous cycle of cell damage and repair caused by chronic skin irritation.

1. INTRODUCTION

In 1982, the American Petroleum Institute (API) reported skin cancer studies on crude oil fractions which showed that atmospheric distillates boiling between 49-371°C (light and middle distillates) produced skin tumours in mice after repeated application. (King, R.W. et al, 1984; Lewis, S.C. et al, 1984)

Some of the fractions tested contained polycyclic aromatic compounds (PACs), and it was thought that these constituents might have been the cause of the tumour development, acting via a genotoxic mechanism. Other fractions, however, e.g. straight-run kerosine and naphtha, had either very low or undetectable amounts of PACs and it was considered unlikely that a genotoxic mechanism was responsible for the tumour development with these samples. Severe skin irritation was noted in the studies reported by the API and it was thought that this was likely to have influenced skin tumour development.

The possibility of different mechanistic pathways leading to skin tumour formation following treatment with middle distillates complicates hazard classification (as required by the EU Dangerous Substances Directive) and human health risk assessment. Consequently, CONCAWE proposed further studies on middle distillates to investigate and clarify the relationships between their PAC content, skin irritation and skin carcinogenicity. A three phase programme was developed and this is described below.

Separate reports on phases 1 (CONCAWE, 1991) and 2 (CONCAWE, 1993; CONCAWE, 1996) are available. The present report provides an overview of the whole programme.

2. OUTLINE OF PROGRAMME

Middle distillates cover a range of petroleum streams which differ in boiling range and composition. They are prepared in a number of ways depending upon variables such as crude source, intended use, type of processing equipment available, product demand and climate. In recognition of this, CONCAWE identified 10 high tonnage middle distillates viz. a hydrotreated naphtha/white spirit, five kerosines and four gas oils, which covered the range of products manufactured in Europe. Thus, the major middle distillate streams to which skin exposure may occur were included (**Table 1**). The intention was to examine these middle distillates in a structured testing programme, ultimately testing a small number of samples in a long-term bioassay for skin carcinogenicity.

The selection of samples for each phase was made on the basis of results from the previous phase. Bulk samples of each distillate were collected and stored under nitrogen at the beginning of the programme and sub-sampled when required. To ensure there was no deterioration during the programme, periodic samples were taken and analysed. No deterioration was detected over a period of almost 10 years.

The main features of each of the programme phases are summarised below.

Phase 1: Short-term tests on 10 middle distillate samples including: analytical studies, *in vitro* genotoxicity studies (modified Ames test), measurement of early changes in skin cells predictive of carcinogenic potential (nuclear enlargement assay, sebaceous gland suppression test) and a description of early histopathological changes in skin from animals treated with selected middle distillates for up to 6 weeks.

Phase 2: Determination of the maximum tolerated dose (MTD) using 5 of the middle distillates and selection of a dosing regime appropriate for long term studies.

Phase 3: A two year skin carcinogenesis bioassay in mice for 3 of the middle distillates.

Table 1: Sample details and testing strategy

Sample No.	Short description	Processing	CAS Number	Phase 1 *	Phase 2 *	Phase 3 *
md-1	Naphtha / white spirit	straight run, hydrotreated	64742-48-9	√	√	
md-2	Kerosine	straight run, wet treated	8008-20-6	√		
md-3	Kerosine	straight run, hydrotreated	64742-81-0	√	√	√
md-4	Kerosine	straight run, hydrotreated	64742-81-0	√		
md-5	Kerosine	straight run, no treatment	8008-20-6	√		
md-6	Gas oil	straight run, hydrotreated	64742-46-7	√	√	√
md-7	LCO	hydrotreated, catcracked light cycle oil	64741-59-9	√	√	√
md-8	Kerosine	blend - straight run, hydrotreated (30%) and hydrocracked (70%)	64742-81-0 / 64741-77-1	√	√	
md-10	Gas oil	blend - straight run (50%) and cat cracked LCO (50%) (blend of samples 6 and 7)	64742-46-7 / 64741-59-9	√		
md-11	Gas oil	commercial gas oil containing 20% cracked material	68334-30-5	√		

key: * : (√) Indicates that this sample was included in phase 1, 2 or 3.

3. RESULTS

3.1. PHASE 1

Ten middle distillates were included in this phase (for sample details, see **Table 1**). Key findings from studies in this phase are summarised in **Table 2** together with details of the content of 3-7 ring PACs for each middle distillate sample as determined by a Mobil method described elsewhere. (Roy, T.A. et al, 1988)

In the modified Ames test, neither the hydrotreated naphtha/white spirit (md-1) nor the kerosine samples (md-2, md-3, md-4, md-5 and md-8) were mutagenic. Some of the gas oils, particularly md-7 and md-10 were mutagenic. The level of activity appeared to relate to their content of 3-7 ring PACs, with the cracked gas oil (LCO) having the highest PAC content and being most active. (CONCAWE, 1991)

Repeated application of middle distillates to the shaved backs of mice caused severe localised skin damage, followed by epidermal hyperplasia. With all samples, effects were well advanced within 1 week (after 3 applications). The distillate with the lowest boiling point (naphtha/white spirit) was the most irritating to skin, causing widespread epidermal necrosis. With the higher boiling kerosines and gas oils, inflammation of skin and necrosis of hair follicles occurred first, followed by degenerative changes. These effects were least severe with the gas oils and most severe with the kerosines.

Results from the nuclear enlargement assay and the sebaceous gland suppression test were inconclusive. As no firm conclusions could be drawn from these specific studies, they will not be discussed further.

In this phase of the programme, all middle distillates caused severe skin irritation when applied repeatedly to mouse skin, but only those containing measurable amounts of 3-7 ring PACs (mainly those containing cracked feed stocks) showed significant mutagenic activity.

In conclusion, the findings from phase 1 were compatible with the assumption that when middle distillates are applied repeatedly to the skin, continual irritation and repair may play a role in the development of skin tumours.

Table 2: Summary of key findings from studies conducted in phase 1 of the programme

Sample description		PAC content (% wt 3-7 rings)	Mutagenicity index *	Nuclear enlargement test	
				Activity	Rel. activity **
md-1	Naphtha / white spirit	ND	0	inactive	1
md-2	Kerosine	ND	0	active	5
md-3	Kerosine	ND	0	active	5
md-4	Kerosine	ND	0	? weak	3
md-5	Kerosine	0.4	0	inactive	2
md-6	Gas oil	1.3	1.0	active	7
md-7	LCO	8.7	14	***	-
md-8	Kerosine (blend)	0.1	0	active	4
md-10	Gas oil (blend)	6.1	7.9	active	8
md-11	Commercial Gas oil	1.0	1.2	***	-

key: * as measured by modified Ames assay (Blackburn, G.R. et al, 1986)
** relative activity in this test, 1 = least active, 8 = most active
*** not possible to score nuclear size due to excessive necrosis.
ND not detected

3.2. PHASE 2

The five samples selected for this phase were a hydrotreated naphtha/white spirit (md-1), a hydrotreated kerosine (md-3), a blended kerosine (md-8), a straight run gas oil (md-6) and a cracked gas oil (md-7).

All samples were tested in a 13 week dermal study. (CONCAWE, 1993) Samples were applied, either undiluted or diluted in acetone or solvent refined mineral oil to the shaved backs of mice, once or twice a week. Skin irritation was assessed visually on a weekly basis and by measurement of dermal myeloperoxidase activity and histopathological examination at termination.

Consistent with the findings in phase 1, all middle distillates caused moderate or severe skin irritation when applied undiluted, irrespective of volume or dosing frequency (100 µl once per week, or 50 µl twice per week). Dilution in acetone (75, 50, 25%) did not significantly alter the degree of irritation, whereas dilution in mineral oil resulted in a clear concentration-related reduction in skin irritation. With an application regime of 50 µl, twice per week, all middle distillates at 75 % in mineral oil produced more than slight skin irritation; at 50 % all samples caused slight or slight to moderate irritation. At 25 %, the kerosines did not produce evidence of skin irritation, whilst the naphtha/white spirit and the gas oils caused only slight irritation. It was thus concluded that middle distillates could be applied repeatedly to mouse skin without causing significant irritation, but that they would require dilution in mineral oil to approximately 25% by volume.

To define the maximum amount of middle distillate that could be applied per week, a further study was conducted. Two kerosines (a straight run, and a blend of straight run and cracked) were applied repeatedly to mouse skin at 25 or 50 % (v/v) in mineral oil over a period of 6 weeks. Neither sample produced more than slight irritation (desquamation) when applied 4 or 7 times per week. This established that it was possible to apply an amount of middle distillate diluted to 25% in mineral oil, over a period of 7 days, which resulted in the same total weekly dose as applying undiluted distillate twice per week (the application rate normally used in a skin painting study).

To confirm that dilution in mineral oil did not reduce dermal penetration of the middle distillate, an absorption study was carried out. Mice were exposed dermally to neat or diluted middle distillates in mineral oil containing ^{14}C-tetradecane and ^{14}C-naphthalene and the levels of absorbed radioactivity measured. The results of two separate studies with naphtha/white spirit and a kerosine blend showed that penetration through the skin was not reduced by dilution in mineral oil, even though such dilution resulted in an increase in viscosity of the applied dose.

From the studies conducted in phase 2, it was concluded that it was possible to investigate the role of skin irritation in skin tumour formation in mice in a 2 year carcinogenicity study. The following three dosing regimes were adopted for phase 3 to ensure that all groups treated with the same sample received the same amount of middle distillate per week, irrespective of dilution rate:

- 100%, applied twice per week (expected to cause irritation).
- 50%, applied 4 times per week (expected to cause slight irritation over the study period).
- 28.5%, applied 7 days per week (expected to be non-irritating).

3.3. PHASE 3

Three of the middle distillates examined in previous phases were selected for further examination in a 2 year skin painting study. (CONCAWE, 1996) These were:

- Straight run, hydrotreated kerosine (SRK), sample md-3
- Straight run, hydrotreated gas oil (SRGO), sample md-6
- Cracked gas oil (light cycle oil, LCO), sample md-7

The study was conducted in accordance with current GLP Guidelines. Animals were housed singly, under controlled environmental conditions, and food/water was provided ad-libitum. Body weight and food consumption were monitored regularly. Skin irritation was assessed daily, using a numerical scoring scheme. Animals were examined regularly for evidence of dermal growths or other signs of toxicity. All animals were sacrificed, either when considered moribund, suspected of having a carcinoma or after 104 weeks treatment and were subject to detailed necropsy examination. Skin masses, tumours and samples of treated and untreated skin were retained and processed for histopathological examination.

Groups of 50 male mice (C3H) were treated with one of the distillates, either undiluted or diluted in a highly refined, non-irritating mineral oil, according to the dosing protocol established in phase 2 (see Section 3.2 and **Table 3**). Additionally, a negative control group of 50 animals was treated 7 days per week with the mineral oil used as diluent. This mineral oil had been shown previously to be non-carcinogenic and non-irritating in a mouse skin carcinogenicity bioassay. (McKee, R.H. and Freeman, J.J., 1993) A positive control group was included in which mice were treated with 5% heavy clarified oil (HCO) twice per week (samples of HCO have previously been shown to be both highly mutagenic in the modified Ames test and carcinogenic in mouse skin painting studies). (CONCAWE, 1989)

Table 3: Two year skin painting study. Experimental design

Test Group No.	Test material	Diluent	conc. of test material (%)	dose (μl)	doses / week	animals / group
1	mineral oil *	none	0	35	7	50
2	HCO	mineral oil	5	50	2	50
3	SRK	none	100	50	2	50
4	SRK	mineral oil	50	50	4	50
5	SRK	mineral oil	28.5	50	7	50
6	SRGO	none	100	50	2	50
7	SRGO	mineral oil	50	50	4	50
8	SRGO	mineral oil	28.5	50	7	50
9	LCO	none	100	50	2	50
10	LCO	mineral oil	50	50	4	50
11	LCO	mineral oil	28.5	50	7	50

key: * CAS No. 64742-54-7, viscosity approximately 20 cSt at 40°C.

Treatment with middle distillates did not significantly affect growth rates when compared to controls.

With regard to survival (**Figure 1**), the HCO-treated positive control group had the lowest survival rate. All animals in this group had been sacrificed by week 60 and, for all but three, this was because of the presence of tumours. Half of the negative control (mineral oil) group had died by week 80 and by the end of the study only 2 animals remained.

The pattern of mortality in all middle distillate treated groups was similar to that of the mineral oil control group, with slightly higher mortality in groups treated with the higher concentrations of middle distillate (50% or 100%). Groups treated with undiluted kerosine, 50% kerosine, or undiluted cracked gas oil had the lowest survival rate.

Dermal irritation was assessed throughout the study and a mean irritation index, (Freeman, J.J. et al, 1990) was calculated according to the scheme outlined in **Table 4**. The irritation indices throughout the study are shown graphically in **Figure 2** and a comparison of irritation indices for each material is shown in **Figure 3**. Treatment with neat kerosine or neat straight run gas oil caused moderate to marked irritation. For these materials, only slight irritation was seen in the 50% groups, whilst the level of irritation at 28.5% was barely perceptible, and occurred in only a few animals.

In contrast, cracked gas oil (LCO) caused skin irritation in all treated animals. The severity of response was related to concentration of LCO. The neat material caused moderate to marked skin reactions, 50% caused moderate irritation and 28.5% provoked only a slight response.

Table 4: Dermal irritation scoring scheme [(a)]

SLIGHT (1)	MODERATE (2)	MARKED (4)
Slight erythema [(b)]	Moderate erythema [(b)]	Extreme erythema [(b)]
Slight oedema	Moderate/marked oedema	Eschar
Desquamation	Atonia	Exfoliation
	Cracking	Fissuring
	Leathery skin	Necrosis
	Pinpoint scabbing	Open sores
	Thickening	Blanching

(a): Numbers (1), (2), (4) represent scores assigned to that group.

NB: the max. score given to any single animal is 4.

$$\text{Irritation index} = \frac{\text{Sum of numeric score in group}}{\text{Number of animals in group}}$$

(b): Erythema was graded as slight, moderate or marked. Oedema was graded as slight or moderate/marked. All other signs of irritation were recorded as present or not present. Animals could exhibit several signs of irritation at one time.

There were no significant treatment-related gross findings at necropsy other than skin irritation and/or tumour development at the site of application. Skin masses and/or tumours developed in several groups. The number of tumour and types of tumours which developed are summarised in **Table 5**.

Table 5: Skin tumour incidence in two year study

Test Group No. (see **Table 3**)	1	2	3	4	5	6	7	8	9	10	11
Test material	min. oil	HCO	md-3 (SRK)			md-6 (SRGO)			md-7 (LCO)		
Middle distillate Conc. (%)	0		100	50	28.5	100	50	28.5	100	50	28.5
group size	50	50	50	50	50	50	50	50	50	50	50
Number of mice examined	50	50	50	49	50	50	50	50	50	50	50
Number of mice with tumours	0	47	12	0	0	4	0	1	1	17	7
Number of days to first tumour	NMO**	217	441	NMO	NMO	554	NMO	644	651	266	301
Tumour types											
Carcinoma, basal cell	0	0	0	0	0	1	0	0	0	0	0
Carcinoma, spindle cell	0	0	1/2*	0	0	0	0	0	0	0	0
carcinoma, squamous cell	0	42/73*	7	0	0	1	0	1	1	8/9 *	3
fibrosarcoma	0	0	3/5*	0	0	0	0	0	1	4	0
keratoacanthoma	0	3	0	0	0	0	0	0	0	0	0
melanoma	0	0	0	0	0	0	0	0	0	1	0
papilloma	0	37/88*	6	0	0	3/4*	0	0	0	10/13*	4

footnote: * number of animals with specified tumour / total number of tumours per group.
** NMO = No Masses Observed

Benign and/or malignant skin neoplasms developed at the site of application in several groups (see below and **Table 5**), but not in groups treated with mineral oil vehicle control, kerosine (50 %, 28.5%) or straight run gas oil (50 %). The HCO positive control group had the highest skin tumour incidence with 47 mice developing a total of 164 tumours (88 benign, 76 malignant).

Of the 3 groups treated with kerosine, only the group treated with undiluted material developed skin tumours. Twelve animals developed a total of 20 tumours (6 benign, 14 malignant).

The pattern of treatment-related tumour incidence was similar with the straight run gas oil. Four mice developed a total of 6 tumours (4 benign, 2 malignant). There were none in the other groups except a single squamous cell carcinoma in the group which received the 28.5% concentration.

In contrast, tumours occurred in all groups treated with cracked gas oil. In the group treated with undiluted material, 1 animal developed tumours (a squamous cell carcinoma and a fibrosarcoma). At a concentration o f 50%, 27 tumours (13 benign, 14 malignant) were recorded in 17 animals. There were 13 papillomas, 9 squamous cell carcinomas, 4 fibrosarcomas and a single melanoma. At a concentration of 28.5%, 7 animals developed a total of 7 tumours, 3 with squamous cell carcinomas and 4 with papillomas.

4. DISCUSSION

The objective of phase one of the programme was to chemically characterise the selected middle distillates, assess their mutagenic activity and study their skin irritation potential. The results showed that all the undiluted middle distillates were severely irritating when applied repeatedly to the skin. The lower viscosity materials caused the most severe effects, which was presumed at least in part to be due to the ability of these materials to penetrate more rapidly and deeply into the skin.

The results of the bacterial mutagenicity studies demonstrated that the straight-run middle distillates which contained low or undetectable levels of PACs were not mutagenic whereas those containing significant levels of 3-7 ring PACs were mutagenic. The mutagenic activity of these samples appeared to be correlated with their 3-7 ring PAC content. This finding is consistent with similar observations with other middle distillates which have been described by Jungen et al. (Jungen, H. et al, 1995; Deininger, G. et al, 1991) Although several methods for the determination of PACs in petroleum fractions are available. The method described by Roy, T.A. et al, (1988) was used in these studies.

Results from the second phase of the programme demonstrated that skin irritation could be reduced to minimal levels if the middle distillates were diluted in highly refined mineral oil. It was also demonstrated that such dilution did not alter the skin absorption of two surrogate molecules as representative of the middle distillates. These findings allowed a dosing regime to be selected for the third phase of the programme.

In the third phase of the programme, a straight-run kerosine, a straight-run gas oil and a cracked gas oil were tested in a two year mouse skin painting study. Each of these middle distillates was applied to the skin using three different dosing regimes, such that for each of them, skin carcinogenicity could be assessed at an irritant dose level, a non-irritant dose level and at a dose level which caused minimal skin irritation. To aid the interpretation of this study, relevant results from the first and third phases of the programme are summarised in **Table 6** and tumour incidence and the occurrence of skin irritation are compared in **Figure 3** .

Table 6: PAC content, mutation index and tumour incidence of test and control materials

Test material	**mineral oil**	**HCO ****	**Straight run kerosine**			**Straight run gas oil**			**Light cycle oil ****		
middle distillate conc. (%)	0	5	100	50	28.5	100	50	28.5	100	50	28.5
3-7 ring PAC (wt.%)	-	-	0	-	-	1.3	-	-	8.7	-	-
mutation index	-	66.1***	0	-	-	1.0	-	-	14.0	-	-
mice examined	50	50	50	49	50	50	50	50	50	50	50
mice with tumours	0	47	12	0	0	4	0	1	1	17	7
Tumour summary:											
mice with malignant tumours *	0	45	11	0	0	2	0	1	1	13	3
total malignant tumours *	0	76	14	0	0	2	0	1	2	14	3
mice with benign tumours *	0	37	6	0	0	3	0	0	0	10	4
total benign tumours *	0	88	6	0	0	4	0	0	0	13	4
Number of all tumours/group	0	164	20	0	0	6	0	1	2	27	7

footnotes:
* Malignant tumours include carcinomas, fibrosarcoma, keratoacanthoma and melanoma (see **Table 5**). Benign tumours - papillomas.
** HCO = Heavy clarified oil. LCO = Light cycle oil (cracked gas oil).
*** Mutagenicity index determined by supplier of sample and not as part of the CONCAWE programme.

For the straight run kerosine, skin tumours only developed in the group of animals in which substantial skin irritation occurred during the study. Since no PACs were detected in the straight run kerosine it is concluded that the occurrence of tumours is likely to have been caused by a non-genotoxic mechanism. This conclusion is consistent with reports by others (Ingram, A.J. and Grasso, P., 1991) that lighter middle distillates are tumour promotors but not initiators and furthermore that skin irritation plays an important role in skin tumour development.

The findings were similar for the straight run gas oil. This material caused a significant increase in the number of tumours only in the presence of skin irritation, and it is thus concluded that the mechanism is unlikely to be a genotoxic one. Again, this finding is consistent with results reported previously (Jungen, H. et al, 1993) that gas oils which contain undetectable or low levels of PACs may be tumour promotors but do not possess tumour initiating activity.

The cracked gas oil contained measurable quantities of PACs and caused the development of skin tumours even at dose levels which did not cause appreciable skin irritation. For this material it is concluded that tumour development was due to a genotoxic mechanism. It was surprising that fewer tumours developed in the group of mice receiving the highest concentration of cracked gas oil. However, this group also showed the most severe skin irritation and it is possible that undiluted cracked gas oil caused a sufficient degree of cytotoxicity to inhibit cell repair and also skin tumour formation.

It is concluded that frequent application of middle distillates that contain undetectable or low levels of PACs to mouse skin will produce skin tumours if accompanied by moderate to marked skin irritation. The mechanism is not considered to involve a direct genotoxic process, but rather may result from frequent cell damage and repair. When irritation does not occur, these same middle distillates do not give rise to tumours.

5. REFERENCES

Blackburn, G.R. et al (1986) Predicting carcinogenicity of petroleum distillation fractions using a modified *Salmonella* mutagenicity assay. *Cell Biol Toxicol 2, 1, 63-84*

CONCAWE (1989) Review of the toxicity of catalytically cracked clarified oil. Report No. 89/56. Brussels: CONCAWE

CONCAWE (1991) Middle distillates - a review of the results of a CONCAWE programme of short-term biological studies. Report No. 91/51. Brussels: CONCAWE

CONCAWE (1993) Middle distillates programme, phase 2: overview of the results of a 13-week skin-painting study. Report No. 93/55. Brussels: CONCAWE

CONCAWE (1996) Two year dermal carcinogenicity study of middle distillates in mice. Project Number 117811A. Study conducted by Exxon Biomedical Sciences Inc. Brussels: CONCAWE

CONCAWE (1996) Middle distillates - an overview of phase 2 of the CONCAWE toxicology programme. Report No. 95/51. Brussels: CONCAWE

Deininger, G. et al (1991) Middle distillates. Analytical investigations and mutagenicity studies. Research Report No. 412-1. Hamburg: DGMK

Freeman, J.J. et al (1990) A 90-day toxicity study of the effects of petroleum middle distillates on the skin of C3H mice. *Toxicol Ind Health 6, 3/4, 475-491*

Ingram, A.J. and Grasso, P. (1991) Evidence for and possible mechanisms of non-genotoxic carcinogenesis in mouse skin. *Mutation Research 248, 333-340*

Jungen, H. et al (1993) Middle distillates. Dermal initiation/promotion study. Research Report No. 412-2. Hamburg: DGMK

Jungen, H. et al (1995) Studies on the tumor initiation/promotion potential of six middle distillates (MDs) in mouse skin. *Fund Appl Toxicol 27, 114-120*

King, R.W. et al (1984) Skin carcinogenic potential of petroleum hydrocarbons: Separation and characterization of fractions for bioassay. In: MacFarland, H.N. et al (eds). Advances in modern environmental toxicology. Volume VI: Applied toxicology of petroleum hydrocarbons, p. 123-138. Princeton NJ: Princeton Scientific Publishers

Lewis, S.C. et al (1984) Skin carcinogenic potential of petroleum hydrocarbons: Crude oil, distillate fractions and chemical class subfractions. In: MacFarland, H.N. et al (eds). Advances in modern environmental toxicology. Volume VI: Applied toxicology of petroleum hydrocarbons, p. 139-150. Princeton NJ: Princeton Scientific Publishers

McKee, R.H. and Freeman, J.J. (1993) Dermal carcinogenicity studies of petroleum-derived materials. In: Wang, R.G.M., Knaak, J.B. and Maibach, H.I. (eds). Health risk assessment: dermal and inhalation exposure and absorption of toxicants, p. 263-274. Boca Raton FL: CRC Press

Roy, T.A. et al (1988) Estimation of mutagenic and dermal carcinogenic activities of petroleum fractions based on polynuclear aromatic hydrocarbon content. In: Polynuclear Aromatic Hydrocarbons: a decade of progress, p. 809-824. Columbus: Battelle Press

Figure 1 Survival curves for the 2 year study

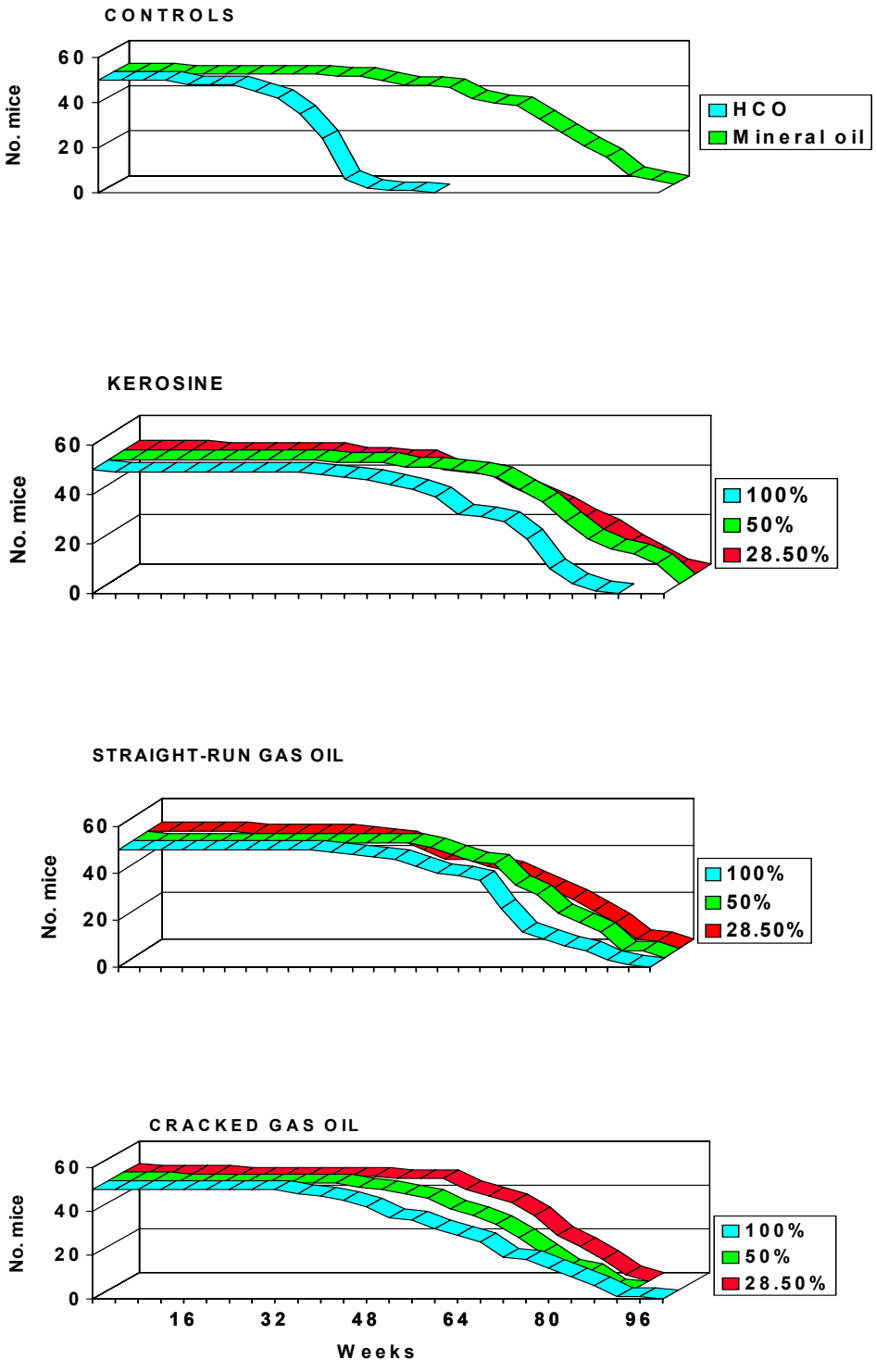


Figure 2 Mean skin irritation indices throughout the 2 year study

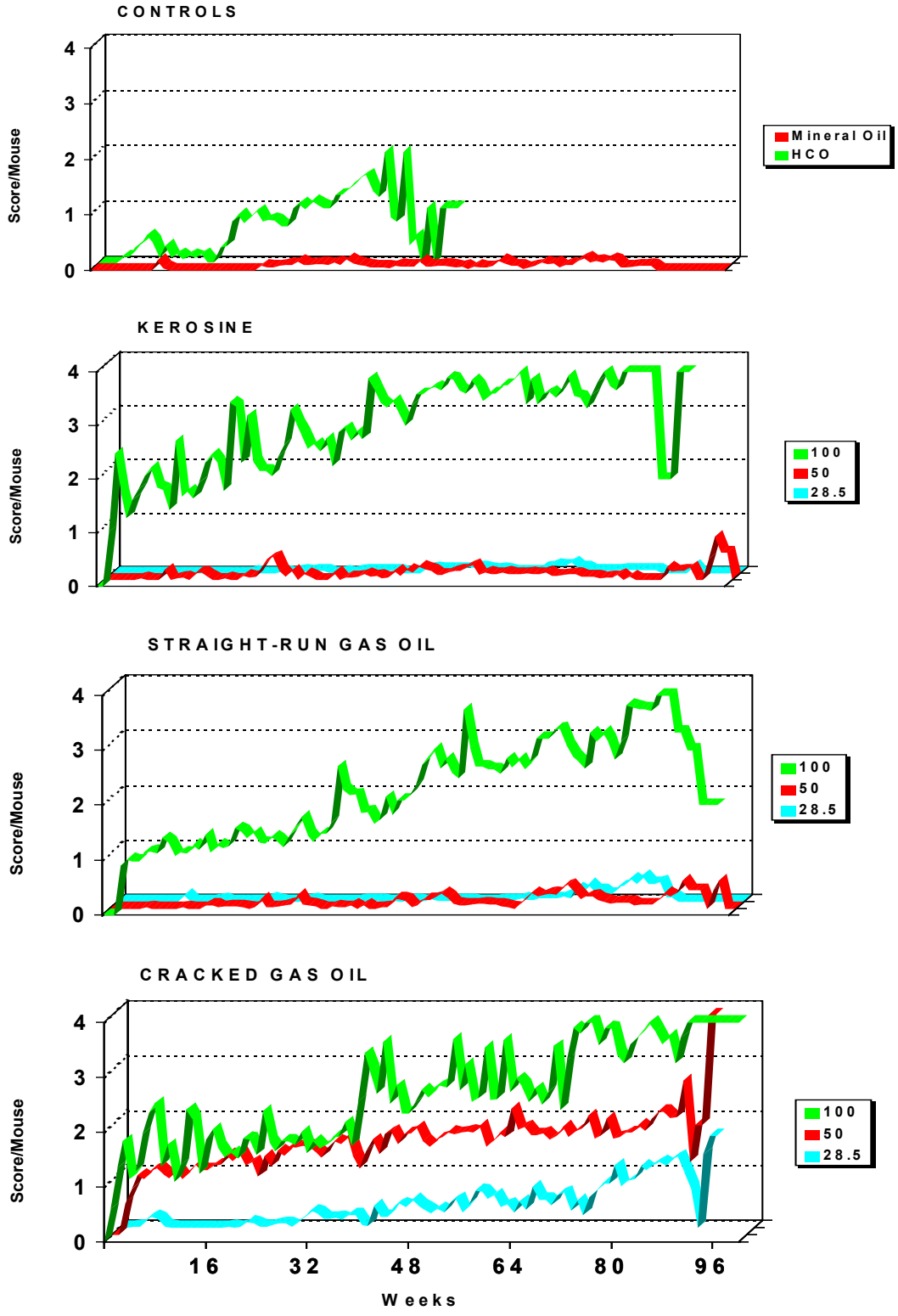
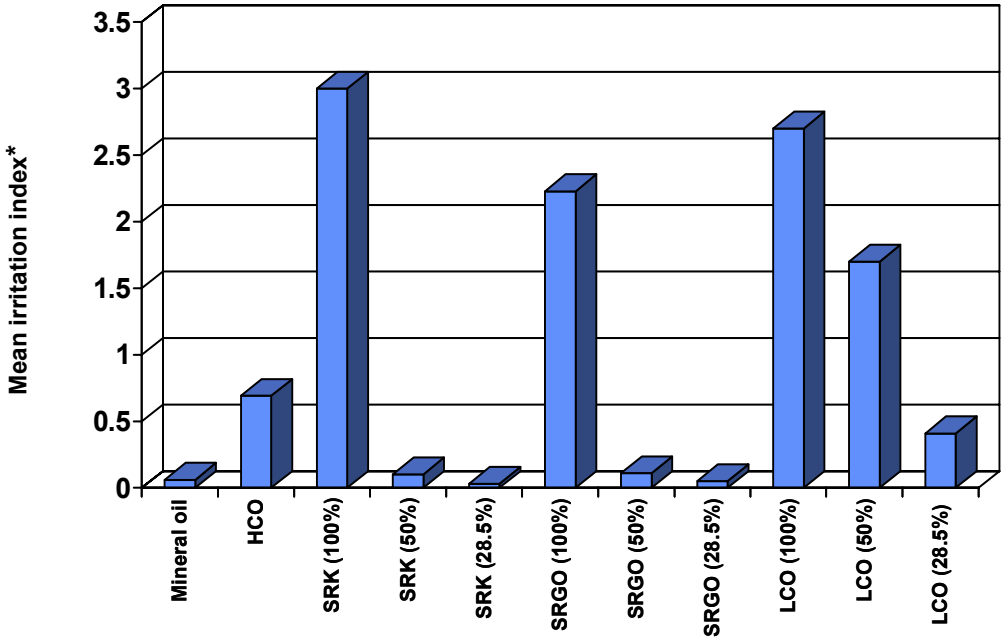


Figure 3 Comparison of mean skin irritation index with tumour incidence



* Mean irritation index calculated as the overall mean of indices throughout the study.