chronic toxicity studies on white oils

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ABSTRACT

This report summarises the results of experimental studies undertaken to investigate the chronic toxicity and carcinogenic potential of two highly refined mineral white oils of medium and high viscosity. The studies were commissioned to support regulatory submissions on the safe use of mineral hydrocarbons in direct food and food contact applications.

Results are presented and discussed and a proposal is made for a No Observed Adverse Effect Level.

KEYWORDS

Animal, chronic toxicity, health effects, rat, white oil, highly refined base oil

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SUMMARY

In response to requests from the European Union Scientific Committee on Food (SCF) and the World Health Organization Joint Expert Committee on Food Additives (JECFA) CONCAWE has sponsored experimental studies to investigate the chronic toxicity/carcinogenic potential of two highly refined white mineral oils, namely P70 (H) and P100 (H).

The studies involved feeding rats for a period of 2 years on diets containing white oil at levels of 60, 120, 240 or 1200 mg/kg/day. The study design also included groups of animals that were fed for 12 months, followed by a further 12 months recovery period, to assess reversibility of any changes.

Treatment did not have any biologically significant effect on the animal survival. There were no clinical signs that were considered treatment-related, nor were there any toxicologically significant effects on body weight, food consumption, food conversion efficiency, or ophthalmology. There was no evidence of treatment-related effects on the haematology, serum chemistry or urinalysis parameters investigated. Neither material caused any significant chronic toxicological effects or neoplastic findings that were considered related to treatment.

At the end of the treatment period, findings for both materials were:

Liver: - presence of tissue mineral hydrocarbon and histopathological changes (cystic degeneration and angiectasis, and portal vacuolisation).

Mesenteric Lymph Nodes: - increased lymph node weight, possible presence of tissue mineral hydrocarbon and increased severity of histiocytosis.

The toxicological significance of these findings is discussed. These marginal, non-neoplastic, histopathological changes were observed, in both studies, together with increased hepatic levels of mineral hydrocarbon, in all dose groups.

The findings were not considered biologically significant however since they were not accompanied by progressive pathology and there was no impact on animal survival. Furthermore, there was evidence to suggest that both the histological lesions and the storage of mineral hydrocarbon (MHC) in the liver were reversible. It is concluded therefore that the No Observed Adverse Effect Level (NOAEL) in both studies was 1200 mg/kg/day.

1. INTRODUCTION

The health hazards of food-grade petroleum-derived white oils and waxes have been evaluated in a number of experimental animal toxicity studies. In a 90-day white oil feeding study, some inflammatory effects (referred to as granulomatous or histiocytic lesions) were observed in the liver and lymph nodes of Fischer 344 (F344) rats [1]. Subsequent 90-day toxicity studies, sponsored by CONCAWE and others, on a wide range of food-grade white oils and waxes confirmed that lower molecular mass (< 500 g/mol) and viscosity (< 11 mm^2/s at 100 °C) white oils and paraffin waxes produce these inflammatory effects, whereas higher molecular weight and higher viscosity white oils and waxes showed minimal to no inflammatory effects in the same organs [2,3]. In a long-term (2 years) study conducted by the Japanese National Institute of Health Sciences [4], a medium viscosity, high molecular weight white mineral oil (P70 mixture) showed no evidence of chronic toxicity or carcinogenic effects. The only significant finding was an inflammatory change in the lymph nodes, which is consistent with that seen in 90-day studies with other white oils. The Japanese study found no shortening of lifespan, no cancers or tumours, nor any other adverse effects. Unfortunately the test material used in this study was not sufficiently well characterised to enable the results to be extrapolated to the range of white oils marketed in Europe.

In 1995 the European Union Scientific Committee on Food (SCF) and the Food and Agricultural Organization of the United Nations (FAO) and the World Health Organization (WHO) Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated white mineral oils and waxes and recommended acceptable daily intake levels (ADIs) for some white oils used as food additives. P70 (H) and P100 (H) were among the oils evaluated, and SCF derived a temporary group ADI of 0-4 mg/kg body weight for these products [5]. JECFA applied a similar approach in deriving their ADIs of 0-1 mg/kg body weight (temporary) for P70 (H) white oil and 0-20 mg/kg body weight for P100 (H) white oil [6].

To facilitate further evaluation of the temporary ADIs assigned, SCF and JECFA requested further data be generated on a P70 (H) oil. The present studies were initiated in response to requests for a two-year chronic toxicity/carcinogenicity study on a P70 (H) oil, including a 12-month reversibility phase; a study on a P100 (H) oil was also commissioned to provide additional data to help in the overall evaluation of the systemic toxicity of white oils. The studies were sponsored by CONCAWE, conducted at the Exxon Biomedical Sciences Laboratory and were initiated in October 1997.

This report summarises the results from the two studies [7,8] and provides CONCAWE's opinion on the toxicological significance of these data.

The results of these studies were submitted to JECFA in 2001/2002 for further evaluation. At the 59^{th} meeting of JECFA (June 2002) the temporary ADI for Class I medium and low viscosity oils was removed and a new permanent ADI of 0-10 mg/kg agreed for these oils. The ADI for high viscosity oils remained at 0-20 mg/kg.

2. MATERIALS AND METHODS

2.1. TEST MATERIAL DETAILS

Samples of white oil grades P70 (H) and P100 (H) were obtained for testing. These materials were representative of currently marketed products. Details are included in **Appendix 1** to this report.

2.2. STUDY OUTLINE

The two studies were conducted to assess the chronic and carcinogenic effects of P70 and P100 white oils in male and female F344 rats, following two-year dietary exposure at different dose levels. The F344 rat was used, based on a SCF/JECFA requirements and results of previous studies, which demonstrated that this strain was sensitive for the findings under investigation. The study design also included an evaluation of the reversibility or persistence of biological effects during a 12-month recovery period, following a 12-month period of exposure.

Each study included five treatment groups: a control group and groups exposed to 60, 120, 240, and 1200 mg/kg/day of the test material. The dose levels were based on those used in previous 90-day studies with similar materials. In the main [24 month] study, each treatment group comprised 50 male and 50 female animals, with an additional 30 male and 30 female animals to accommodate the reversibility phase. In addition, small groups, of five female animals each, were added to allow evaluation of tissue hydrocarbon levels at 3, 6 12, 18 and 24 months [**Table 1**]. The choice of female animals was based on results obtained in previous work where higher hydrocarbon levels were reported for females than for males [3].

The test materials were administered in the diet, at concentrations set to achieve the target dose on a mg/kg/day basis. Toxicological parameters investigated during the study included body weight, food consumption, clinical observations, serum chemistry, haematology, ophthalmology, urinalysis, and organ weights. Tissue mineral hydrocarbon analysis was performed on liver, kidneys, mesenteric lymph nodes and spleen from female animals at various time points throughout the study. Detailed histopathological examination of a comprehensive range of tissues, with particular emphasis on liver, mesenteric lymph nodes, spleen, and kidneys, was also conducted.

Table 1 General study design - Experimental groups

Target dose group (mg/kg/day)	Animals total		Main study (up to 24 month exposure)		Recovery study (12 month exposure with recovery)		Animals for MHC[*)] analysis of tissues
Sex	M	F	M	F	M	F	F
0	80	111	50	50	30	30	31
60	80	105	50	50	30	30	25
120	80	105	50	50	30	30	25
240	80	105	50	50	30	30	25
1200	80	127	50	50	30	30	47

[*)] MHC: mineral hydrocarbon

3. RESULTS

Detailed results of the two studies are given in the final laboratory reports [7,8]. The following text summarises the main points of scientific interest.

Dietary administration of P70 (H) and P100 (H) to F344 rats for 24 months did not have any biologically significant effect on the survival of either sex (**Table 2**). There were no clinical signs that were considered treatment-related, nor were there any toxicologically significant effects on body weight, food consumption, food conversion efficiency, or ophthalmology. There was no evidence of treatment-related effects on the haematology, serum chemistry or urinalysis parameters investigated.

There were no treatment-related observations at gross necropsy. Treatment with the two test materials did not result in any significant chronic toxicological effects or neoplastic findings that were considered related to treatment. However, there were some findings in both studies that merit further discussion and these are addressed below.

Table 2 Percentage survival until end of study: P70 and P100

Target dose group (mg/kg/day)	Main study P70		Main study P100		Recovery study P70		Recovery study P100	
Sex	M	F	M	F	M	F	M	F
0	68	74	56	78	75	75	65	80
60	68	72	74	82	85	65	45	75
120	74	74	66	78	75	65	55	95
240	52	76	58	80	70	60	70	100
1200	64	70	66	64	70	80	70	90

3.1. P70 (H)

The findings with this material that need further consideration were: organ weight changes, histopathology of liver, mesenteric lymph nodes and blood and presence of mineral hydrocarbons in liver and mesenteric lymph nodes.

3.1.1. Organ weight effects

The only consistent, statistically significant organ weight change seen following treatment with P70 (H) was for the mesenteric lymph nodes.

In males, this occurred in the 1200 mg/kg/day group only, at the 12-month and 24-month sacrifices. The increase was reflected in mesenteric lymph node weight, mesenteric lymph node to body weight ratios, and mesenteric lymph node to brain weight ratios. Mesenteric lymph node weights were not increased in any of the male recovery groups.

In females, mesenteric lymph node weights were increased in all treatment groups at the 24-month sacrifice, reflected by increases in mesenteric lymph node weight, ratio of mesenteric lymph node weight to body weight, and ratio of mesenteric lymph node to brain weight. There was also a statistically significant increase in most of these parameters for 240 and 1200 mg/kg females in the recovery study.

3.1.2. Histopathology

3.1.2.1. Leukaemia

There was an increased incidence of mononuclear leukaemia in female rats at all dose levels (**Table 3**). The incidence, however, was not dose-related and within the historical control range for this strain of rat (14-52%) [10]. There was no increase in the incidence of leukaemia in treated males.

Table 3 Incidence of mononuclear cell leukaemia in P70 (H) treated animals – Main study

Sex	Males					Females				
Target dose group (mg/kg/day)	0	60	120	240	1200	0	60	120	240	1200
Number (#) of animals examined	50	50	50	50	50	50	50	50	50	50
Incidence (%)	26	34	22	20	30	14	30	24	38	28

3.1.2.2. Liver

There were no treatment-related histopathological findings in the liver after 12 months of treatment. After 24 months, there was an increased incidence of angiectasis (focal dilation of the sinusoidal spaces) and cystic degeneration (focal dilated vascular spaces accompanied by eosinophilic debris or necrotic, often ballooned, hepatocytes) in the livers of treated males at all dose levels. Similar pathology was also observed, but at a very low incidence, in control groups and treated females (**Table 4**).

The study pathologist considered these two liver lesions to be related, with cystic degeneration being the initial effect. The dilated sinusoids, characteristic of angiectasis, occurred with the loss of necrotic hepatocytes. The combined incidence of these lesions was significantly increased in males at 60, 120, 240 and 1200 mg/kg; however there was no dose response relationship.

Spontaneous angiectasis is a common finding in the Fischer 344 rat, particularly in males [11]. Therefore the biological significance of these lesions is questionable, particularly since they were of minimal severity, and there was no dose response relationship. Furthermore there was no evidence of proliferation, such as foci of cellular alteration or hepatic or vascular neoplasia.

In recovery group animals there was no evidence of a treatment-related increase in these findings.

Table 4 Incidence of liver pathology for animals surviving 24 months (main and recovery studies) in P70 treated animals

Sex	Main study males					Main study females				
Target dose group (mg/kg/day)	0	60	120	240	1200	0	60	120	240	1200
# Examined	33	33	34	26	31	37	34	37	37	33
Angiectasis	2	11*	7	8*	4	3	1	4	0	1
Cystic Degeneration	5	12*	14	8	17**	0	0	1	1	2
Combined total (and percentage)	7 21%	20** 61%	18* 53%	16** 62%	20** 65%	3 8%	1 3%	5 14%	1 3%	3 9%
Sex	Recovery study males					Recovery study females				
Target dose group (mg/kg/day)	0	60	120	240	1200	0	60	120	240	1200
# Examined	12	17	14	14	14	14	12	13	12	15
Angiectasis	2	3	1	3	5	0	1	1	1	1
Cystic Degeneration	5	5	3	8	5	0	0	0	0	0
Combined total (and percentage)	6 50%	8 47%	4 29%	8 57%	9 64%	0 0%	1 8%	1 8%	1 8%	1 7%

* - Statistically significant at $p \leq 0.05$ by Fischer Exact Test

** - Statistically significant at $p \leq 0.01$ by Fischer Exact Test

There was also an increase in the incidence, and a small increase in severity, of vacuoles in the portal area of the liver in treated males. This finding was difficult to detect but was also observed in control animals. The vacuoles were present in the cytoplasm of macrophages or located extra-cellularly. A smaller increase in severity was noted in treated females. A small increase in incidence of vacuoles was also observed in recovery group males, suggesting incomplete resolution, although this was not dose-related. The study pathologist considered that the vacuoles were not an indication of an adverse effect, but probably reflects the presence of test material following prolonged administration.

3.1.2.3. Mesenteric lymph nodes

The incidence of histiocytosis (infiltrating histiocytes) in the mesenteric lymph nodes after 24 months of treatment was similar in control and all treated groups, but the severity of response was slightly greater in both sexes of all treatment groups compared to controls (**Table 5**). In males, but not females, the slight increase in severity appeared to be dose-related.

In the recovery study, the incidence of histiocytosis was again similar in control and treated groups. Whilst there were some differences in severity of response in treated groups, these were not as large as those seen after 24 months treatment. Neither males nor females showed a dose-related response.

Table 5 Incidence and mean severity of histiocytosis in the mesenteric lymph node for 24-month survivors in P70 treated animals

	Main study			Recovery study		
Target dose group (mg/kg/day)	Examined (#)	Incidence (%)	Mean severity*	Examined (#)	Incidence (%)	Mean severity*
Males						
0	33	85	1.2 ± 0.7	12	100	1.5 ± 0.5
60	33	100	1.6 ± 0.7**	17	94	2.2 ± 1.0**
120	34	97	1.8 ± 0.8***	14	100	2.6 ± 0.6***
240	26	100	2.3 ± 0.8***	14	100	2.4± 0.6***
1200	31	94	2.1 ± 0.9***	14	100	1.6 ± 0.6
Females						
0	37	97	1.6 ± 0.7	14	100	1.9 ± 0.6
60	34	100	2.4 ± 0.6***	12	100	2.3 ± 0.6
120	37	95	2.5 ± 0.9***	13	100	1.9 ± 0.5
240	37	97	2.6 ± 0.7***	12	100	2.6 ± 0.5***
1200	33	97	2.4 ± 0.8***	15	100	2.5 ± 0.6**

* - Severity scale: 1 - minimal, 2 - mild, 3 - moderate, 4 - marked
** - Statistically significant at the $p \leq 0.05$ level
*** - Statistically significant at the $p \leq 0.01$ level

3.1.3. Tissue levels of mineral hydrocarbons (female animals only)

3.1.3.1. Liver

Detectable levels of mineral hydrocarbon were found in the liver of all animals, including controls (**Appendix 2, Table A2.1**). Some individual values were, however, below the practical level of quantitation (PQL, see Glossary). At 3 months the amount was increased at 1200 mg/kg (only group analysed) compared to controls. Thereafter levels increased more slowly. The levels of mineral hydrocarbon were of the same order in the 60, 120, and 240 mg/kg groups at 12 and 24 months, *i.e.* there was no apparent dose-related response. The increase in tissue concentration at 1200 mg/kg was statistically significant compared to control animals from 6 months onwards. The level of mineral hydrocarbons in the other treatment groups was only increased significantly after 24 months.

In recovery animals the concentration of mineral hydrocarbons had decreased substantially six months after cessation of treatment (18-month analysis of 1200 mg/kg/day recovery animals); levels were however still increased compared with controls. After 12 months without treatment (24-month analysis of recovery animals), the levels were essentially similar to those measured in the control group.

3.1.3.2. Mesenteric lymph nodes

Mineral hydrocarbons were not detected in the mesenteric lymph nodes at 3- and 6-month time-points (**Table A2.1 in Appendix 2)**. Some mineral hydrocarbon was found in mesenteric lymph nodes at other time-points, starting at 12 months, however, levels were below the practical limit of reliable quantification.

The results of mesenteric lymph node, mineral hydrocarbon analysis, should be treated with caution in view of the technical difficulties in measuring MHC in very small organs. It is likely that this also contributes to the large variability seen around the mean MHC levels.

3.1.3.3. Spleen

Mineral hydrocarbon was not detected at the 3-month time-point. Some mineral hydrocarbon was found at other time-points, starting at 6-months, however, levels were below the practical limit of reliable quantification (**Table A2.1**).

3.1.3.4. Kidneys

Mineral hydrocarbons were not detected in the kidney at any time-point.

3.2. P100 (H)

The findings with this material that merit further discussion were: organ weight changes, histopathology of liver, mesenteric lymph nodes, pituitary and thyroid and presence of mineral hydrocarbons in liver and mesenteric lymph nodes.

3.2.1. Organ weight effects

The only statistically significant organ weight change seen with P100 (H) was for the mesenteric lymph nodes of females at 1200 mg/kg, after 24 months of treatment. This was reflected as an increase in absolute mesenteric lymph node weight, ratio of mesenteric lymph node weight to body weight, and ratio of mesenteric lymph node to brain weight. There were no differences observed in the lymph node weights of male treated animals, other female treatment groups or recovery group animals.

3.2.2. Histopathology

3.2.2.1. Pituitary

There was an increased incidence of adenoma involving the pars distalis of the pituitary in females at 1200 mg/kg (**Table 6**). The incidence however did not exceed the upper boundary of the historical control range for this strain (30 to 74%) [10]. There was no increase in the incidence of this lesion in males.

Table 6 Incidence of pituitary/pars distalis adenoma in P100 treated animals – Main study

Sex	Males					Females				
Target dose group (mg/kg/day)	0	60	120	240	1200	0	60	120	240	1200
# Examined	50	19	18	21	50	50	9	14	10	50
Incidence (%)	38	26	28	43	40	42	22	50	40	60

3.2.2.2. Thyroid

There was a slight, non-statistically significant increase in the incidence of thyroid C-cell hyperplasia in females at 1200 mg/kg (**Table 7**). The increased incidence was not evident however in those animals surviving to 24 months. This finding was not considered to be treatment related however, as this lesion occurs in untreated rats at a similar incidence. The lesion was morphologically similar to that seen in the untreated control group and the incidence was slightly lower than in the control group of the P70 (H) study.

Table 7 Incidence and severity of thyroid C-cell hyperplasia in P100 treated animals

Sex	Males					Females				
Target dose group (mg/kg/day)	0	60	120	240	1200	0	60	120	240	1200
# Examined	50	20	18	21	50	50	9	14	10	50
Incidence (%)	12	1	6	19	12	14	11	14	20	24
Severity	0.20	0.20	0.06	0.38	0.16	0.20	0.22	0.14	0.30	0.36

3.2.2.3. Liver

There was an increase in the incidence of angiectasis (focal dilation of the sinusoidal spaces) and cystic degeneration (focal dilated vascular spaces accompanied by eosinophilic debris or necrotic, often ballooned hepatocytes) in the liver of males from all dose levels, following 24-months treatment (**Table 8**). Similar lesions were also observed in females, but at a much lower incidence.

The study pathologist considered these two liver lesions to be related, with cystic degeneration being the initial lesion. The dilated sinusoids, characteristic of angiectasis, occurred with the loss of necrotic hepatocytes. Individually, the incidence of these lesions was not statistically significant compared with controls. The combined incidence was significantly increased, but only in 1200 mg/kg treated males that survived to 24 months.

Spontaneous angiectasis is a common finding in the Fischer 344 rat, particularly in males [11]. Therefore the biological significance of these lesions is questionable, particularly since they were of minimal severity and without evidence of proliferation, such as foci of cellular alteration or hepatic or vascular neoplasia.

Livers of recovery group animals were not evaluated because there were no effects noted after 12-months treatment.

Table 8 Incidence of angiectasis and cystic degeneration in the liver of P100 treated animals surviving until study end – Main study

Sex	Males					Females				
Target dose group (mg/kg/day)	0	60	120	240	1200	0	60	120	240	1200
# Examined	28	30	32	29	33	39	41	36	40	29
Angiectasis	1	2	3	5	6	1	1	1	2	2
Cystic degeneration	11	16	16	15	21	0	0	0	2	1
Combined total (and percentage)	11 39%	18 60%	18 56%	18 62%	24* 73%	1 3%	1 2%	1 3%	4 10%	3 10%

* - Statistically significant at $p \leq 0.05$

In addition to the above findings there was a slight, but statistically significant increase in the incidence, and a slight increase in severity, of vacuoles in the portal area of the liver in treated males and females. The vacuoles were present in the cytoplasm of macrophages or located extra-cellularly. This lesion was difficult to detect and occurred also in control animals. The study pathologist considered that the vacuoles were not an indication of an adverse effect, but probably reflects the presence of test material following prolonged administration.

3.2.2.4. Mesenteric lymph nodes

At 24-months there was a slight, increase in the severity of histiocytosis in mesenteric lymph nodes of males and females of all treatment groups (**Table 9**). The difference in severity was small and only statistically significant in female animals that survived 24 months treatment. The severity did not appear to be dose-related and the incidence of this lesion was similar in all treated and control groups.

Table 9 Incidence and severity of mesenteric lymph node histiocytosis (24-month survivors) in P100 treated animals

Sex	Males					Females				
# Examined	28	29	32	29	33	39	41	36	40	29
Incidence (%)	96	100	100	100	94	100	98	100	100	100
Severity*	1.6	2.0	1.9	2.1	2.0	1.9	2.2**	2.5**	2.4**	2.6**

* - Severity scale: 1 - minimal, 2 - mild, 3 - moderate, 4 - marked

** - Statistically significant at the $p \leq 0.01$ level

3.2.3. Tissue levels of mineral hydrocarbons (females only)

3.2.3.1. Liver

Measurable levels of mineral hydrocarbon were found in the liver of all treated groups (**Table A2.2 in Appendix 2**). Control group mean levels were below the Practical Quantitation Limit at 3, 6, and 12-months.

In the 1200 mg/kg group there was an increase of mineral hydrocarbon levels at 3, 6, 12, 18 and 24 months. At three months, tissue levels had increased significantly, compared to controls, but thereafter levels increased more slowly. For other dose groups, tissue levels were elevated at 12 and 24 months, the only time intervals evaluated. Tissue levels did not appear to show a dose-related response.

In recovery animals liver mineral hydrocarbons decreased substantially within six months of cessation of treatment, but levels were still increased compared with controls. After a further six months the amount of mineral hydrocarbons in the liver had essentially returned to control, background levels.

3.2.3.2. Mesenteric lymph nodes

Mineral hydrocarbons were not detected in the mesenteric lymph nodes at the 3-, 6- and 12-month time-points. Detectable levels of mineral hydrocarbon were found in mesenteric lymph nodes at other time-points, starting at 18 months, however, the majority of the individual values were below the practical limit of reliable quantification.

In recovery group animals, mineral hydrocarbon was detected but levels were below the practical limit of reliable quantification.

The results of mesenteric lymph node, mineral hydrocarbon analysis, should be treated with caution in view of the technical difficulties in measuring MHC in very small organs. It is likely that this also contributes to the large variability seen around the mean MHC levels.

3.2.3.3. Spleen

Mineral hydrocarbon was not detected at the 3-month time-point. Some mineral hydrocarbon was found at other time-points, starting at 6 months, however, levels were below the practical limit of reliable quantification. No mineral hydrocarbon was detected in recovery group animals.

3.2.3.4. Kidney

With the exception of an isolated finding of quantifiable mineral hydrocarbons in one 1200 mg/kg/day female at the 6-month interval, mineral hydrocarbons were not detected in the kidney.

4. DISCUSSION

The principal findings in chronic feeding studies with these two mineral oils were similar. Neither material caused any adverse clinical effects, and there were no toxicologically significant effects on mortality, body weight, food consumption/ conversion, ophthalmology, haematology, serum chemistry and urine chemistry. There were no treatment related observations at gross necropsy and no neoplastic findings considered related to treatment; the leukaemia (for the P70 oil), and pituitary and thyroid (for the P100 oil) findings have been discussed previously in the results section.

The only organ weight effect reported was an increase in absolute and relative mesenteric lymph nodes weight. Treatment-related histopathological effects were limited to liver and mesenteric lymph nodes.

A number of findings in these studies were consistent with observations made in previous feeding studies with mineral oils [1,3]. These, together with results of the current studies, suggest liver and mesenteric lymph nodes are the primary target organs.

The 1200 mg/kg/day No Observed Adverse Effect Level (NOAEL) was used by JECFA to confirm an Acceptable Daily Intake (ADI) of 0-20 mg/kg for P100 (H) and 0-10 mg/kg for P70 (H).

4.1. LIVER

Mineral hydrocarbon was found in the liver of rats following prolonged feeding with both P70 and P100 oils. Significant tissue levels were measured after 3 months at the highest dose, but thereafter levels increased more slowly. After 12 and 24 months feeding, significant tissue levels were seen with both materials, in all treatment groups. For both materials, tissue levels returned to normal background levels within 12 months of cessation of treatment.

Both oils caused an increase in the incidence of hepatic effects, after 24 months feeding and in male rats only, described as angiectasis and cystic degeneration. These lesions were not present at 12 months, nor were they observed in recovery group animals. They also occur spontaneously, albeit at a lower incidence, in control animals and there was no evidence of a dose-effect relationship.

The two liver lesions are considered to be related, with cystic degeneration being the primary effect. These liver lesions are not considered to be of biological importance however for the following reasons:

- The lesions were of minimal severity and there was no dose-response relationship,
- There was no evidence of associated proliferative or progressive response,
- The pathologist's experience is that the incidence of these findings in the rat increases with age,
- The incidence of the hepatic findings in these studies was within the normal range reported by the United States National Toxicology Program (NTP) for this strain of rat [11],
- There was no associated increase in mortality or effects on animal well-being.

Prolonged exposure to P70 and P100 was also associated with an increase in the incidence of vacuoles in the portal area of the liver, primarily in males. There was also a slight increase in the severity of this response over time. This finding was difficult to detect, of minimal severity and not associated with any other tissue response such as inflammatory cell reaction. It was observed primarily in male animals and also occurred with a lower incidence and severity in control animals. In test animals the incidence was slightly increased but there was no apparent dose-effect relationship.

This finding was treatment related but was not considered to be an adverse effect due to the lack of any other tissue response, no accompanying clinical-chemistry effects and no apparent impact on the well being of the animals. It is considered to reflect presence of test material following prolonged administration.

4.2. MESENTERIC LYMPH NODES

There was some indication of the presence of mineral hydrocarbons in the mesenteric lymph nodes, particularly during the later stages of these studies. The levels were however below the amount which could be reliably quantified. It is therefore not possible to draw any firm conclusions on the rate of increase in tissue levels or it's reversibility in this organ.

Mesenteric lymph node weights were increased in animals treated with both P70 and P100 oils. With P100, the increase was restricted to the 1200 mg/kg female group and was only seen following 24 months treatment. Female animals treated with P70 showed a treatment related increase in lymph node weight, following 24 months treatment. The increase was also observed in some recovery groups. For P70 males the increase was only seen in the 1200 mg/kg group.

Histopathological examination showed a slight, treatment related increase in the severity of histiocytosis in the mesenteric lymph nodes. This finding was observed with both materials following 24 months of treatment and was still evident in some recovery groups.

Histiocytosis in mesenteric lymph nodes is a common finding in the rat, particularly females, and is also known to be an age related finding. The background incidence in control animals and the similar incidence in treated groups support this. Treated animals showed a slight increase in severity of this finding (from minimal to mild). This finding is not considered to be of toxicological significance for the following reasons:

- The increase in severity was small and was only slightly above that seen in control animals,
- There was evidence of reversibility, but not complete resolution following cessation of treatment,
- The incidence of this finding in test and control groups was similar,
- There were no accompanying clinical or haematological effects related to treatment,
- The finding at 24 months is morphologically similar to that seen in animals examined at 12 months and from previous 90-day feeding studies with mineral oils,

- The slight increase in severity of histiocytosis is consistent with a normal inflammatory cell response to the presence of mineral hydrocarbon,
- The data at 24 months and from the reversibility phase indicate that the finding is not progressive and also that there is no effect on animal well being.

The lesions observed in the mesenteric lymph nodes and liver in F344 rats are different morphologically from changes observed in tissues from human mineral oil users [12,13,14]. The lipogranulomas found in human tissue specimens are considered incidental and inconsequential. Two expert pathology review panels convened during the past decade have confirmed this judgement.

4.3. CONCLUSION

Treatment of rats with mineral white oils for two years with up to 1200 mg/kg/day did not result in any findings of biological significance.

The findings of mineral hydrocarbon accumulation and inflammatory cell reaction seen in some tissues in this, and the previous 90-day studies, are not considered adverse findings for the following reasons:

- there was no impact on survival,
- they were not accompanied by any treatment-related clinical findings or progressive pathology,
- there was evidence to indicate both findings were reversible.

The NOAEL is therefore considered to be 1200 mg/kg/d.

5. GLOSSARY

ADI	-	Acceptable daily intake, amount of a substance, such as a food additive, usually expressed in milligram per kilogram of body weight, that can be ingested daily over a lifetime without appreciable health risk
JECFA	-	The Joint FAO/WHO Expert Committee on Food Additives [FAO: Food and Agricultural Organization of the United Nations; WHO: World Health Organization]
MHC	-	Mineral hydrocarbon, defined as the product of the following analytical procedure: samples of animal tissue or feed are extracted with an organic solvent; the extract is filtered on silica gel to remove polar components; the extract is then analysed via gas chromatography with flame ionisation detection; finally, the chromatogram is compared with a chromatogram of the tested [petroleum-derived] white oil in the solvent and the components that can be recognised as originating from the white oil are quantified and added up
NOAEL	-	No observed adverse effect level: highest tested level at which no adverse effects were observed
P70 (H) white oil	-	White mineral oil, belonging to the group of highly refined base oils, produced from a paraffinic (P) crude source via a hydrotreating (H) process and with a viscosity of 70 mm^2/s at 40 °C. Mineral oil, medium and low viscosity, Class I (JECFA classification)
P100 (H) white oil	-	White mineral oil, belonging to the group of highly refined base oils, produced from a paraffinic (P) crude source via a hydrotreating (H) process and with a viscosity of 100 mm^2/s at 40 °C. Mineral oil, high viscosity (JECFA classification)
PQL	-	Practical Quantitation Limit; varying figure, denoting lowest amount that can be quantified reliably, depending on *inter alia* the analytical detection limit and the amount of available test material
SCF	-	The European Union's Scientific Committee on Food

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APPENDIX 1

TEST MATERIAL PHYSICAL/CHEMICAL INFORMATION

Table A1.1 Selected chemical and physical properties of tested white oils

TEST	METHOD	P70[1]	P100[2]
Density at 15°C, kg/m^3	DIN 51757	868.8	873.5
Refractive index at 20°C	DIN 51423-2	1.4754	1.4778
Kin. viscosity at 40°C, mm^2/s	ISO 3104	70.4	100.3
Kin. viscosity at 100°C, mm^2/s	ISO 3104	8.97	11.3
Viscosity Index VI/VI-E	ISO 2909	101	98
Refractive Intercept (FI)	ASTM D2140 mod	1.0425	1.0426
Viscosity Gravity Constant (VGC)	ASTM D2140 mod	0.799	0.799
Carbon Distribution - C/A (S-corr.), % - C/N (S-corr.), % - C/P (S-corr.), %	ASTM D2140 mod	 0 32 68	 0 32 68
Simulated Distillation (°C) - initial/final boiling point - boiling point at 5%	ASTM D2887 ext	 349/555 418	 399/597 437
Carbon Number at 5% boiling point	from ASTM D2887	C-27	C-29
Molecular mass, g/mol	ASTM D2502	485	520
UV-Absorption (260-420 nm) for White Oils	EUPH 2	Pass	Pass

[1] Mineral oil, Medium and Low Viscosity, Class I (JECFA classification)
[2] Mineral oil, High Viscosity (JECFA classification)

APPENDIX 2

TISSUE MINERAL HYDROCARBON LEVELS

Table A2.1 P70 (H) Mean concentration of mineral hydrocarbons in selected organs (µg/g)

Group	Liver	MLN	Spleen
3 Month			
Control	*273±26*	ND	ND
1200 mg/kg/day	1560±237	ND	ND
6 Month			
Control	315±45	ND	ND
1200 mg/kg/day	1600±167**	ND	*166±19*
12 Month			
Control	320±4	ND	ND
60 mg/kg/day	1100±69	ND	ND
120 mg/kg/day	1190±167	ND	*26±57*
240 mg/kg/day	1140±147	ND	ND
1200 mg/kg/day	1850±458**	*1428±879*	*26±59*
18 Month			
Control	*231±32*	ND	ND
1200 mg/kg/day	1840±390	*4230±2200*	*260±250*
1200 mg/kg/day Recovery	675±79	*1210±840*	*62±89*
24 Month			
Control	414±139	ND	ND
60 mg/kg/day	1430±207**	*2200±1280*	ND
60 mg/kg/day Recovery	449±40	ND	ND
120 mg/kg/day	1220±425*	*2370±1030*	ND
120 mg/kg/day Recovery	506±73	*840±1290*	ND
240 mg/kg/day	1500±256**	*2430±1610*	*125±171*
240 mg/kg/day Recovery	489±115	ND	ND
1200 mg/kg/day	2260±740**	*3790±1660*	*396±242*
1200 mg/kg/day Recovery	585±208	*568±812*	ND

MLN - Mesenteric lymph nodes
ND - Not detected
Values in italics represent the group mean of groups where not all individual values were above the PQL (Practical Quantitation Limit)
* - Statistically significant at the $p \leq 0.05$ level
** - Statistically significant at the $p \leq 0.01$ level

Table A2.2 P100 (H) Mean concentration of mineral hydrocarbons in selected organs (µg/g)

Group	Liver	Spleen	MLN	Kidney
3 Month				
Control	*298±57*[a]	ND	ND	ND
1200 mg/kg/day	891± 82*	ND	ND	ND
6 Month				
Control	*338±103*[a]	ND	ND	ND
1200 mg/kg/day	929± 112**	*253±196*	ND	b
12 Month				
Control	*286±34*[a]	ND	ND	ND
60 mg/kg/day	804± 49*	ND	ND	ND
120 mg/kg/day	817± 51*	*41±93*	ND	ND
240 mg/kg/day	764± 147	ND	ND	ND
1200 mg/kg/day	932± 183*	ND	ND	ND
18 Month				
Control	346± 31	ND	ND	ND
1200 mg/kg/day	1430± 113**	*75±167*	*2160±530*	ND
1200 mg/kg/day Recovery	573± 61**	ND	*1160±774*	ND
24 Month				
Control	424± 57	ND	ND	ND
60 mg/kg/day	1210± 120**	ND	*1322±1293*	ND
60 mg/kg/day Recovery	420± 61	ND	ND	ND
120 mg/kg/day	1360± 257**	ND	*1736±1761*	ND
120 mg/kg/day Recovery	380± 46	ND	*622±1391*	ND
240 mg/kg/day	1400± 343**	ND	*3054±2134*	ND
240 mg/kg/day Recovery	456± 138	ND	ND	ND
1200 mg/kg/day	1360± 395**	*3300±3580*	*3380±831*	ND
1200 mg/kg/day Recovery	511± 56	ND	*306±684*	ND

MLN - Mesenteric lymph node
ND - Not detected
Values in italics represent the group mean value for groups where not all values were above PQL (Practical Quantitation Limit)
* - Statistically significant at the $p \leq 0.05$ level
** - Statistically significant at the $p \leq 0.01$ level
a - Mean value used for statistical analysis even though below PQL
b - One animal with a value of 204