an assessment of the reproductive toxicity of gasoline vapour

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Brussels
November 2000

ABSTRACT

The reproductive toxicity of gasoline vapour in rats was studied in order to generate relevant toxicity information for gasoline in humans via the inhalation route. The two-generation study was designed and conducted according to international guidelines. Exposure levels were 0 and approximately 5000, 10 000 and 20 000 milligram per cubic metre. No treatment-related adverse effects relevant to humans were observed. It was concluded that the No-Observed-Adverse-Effect-Level for reproductive toxicity in rats in this study was 20 000 milligram per cubic metre.

KEYWORDS

Gasoline (CAS 86290-81-5), Vapour Recovery Unit Gasoline (CAS 68514-15-8), Low Boiling Point Naphtha, Reproductive Toxicity

CONTENTS

SUMMARY

Gasoline (CAS 86290-81-5) is a high volume, commercial product. Although numerous toxicology studies have been conducted, the potential for reproductive toxicity has not been directly assessed. Accordingly, a two-generation reproductive toxicity study in rats was conducted to provide base data for health hazard assessment and risk characterization. The material tested, vapour recovery unit gasoline (68514-15-8), is the volatile fraction of gasoline and the material with which humans are most likely to come in contact. The study was designed and conducted according to international guidelines. The test animals were exposed by inhalation at concentrations up to 20 000 mg/m^3, which is the highest level considered to be safe to test (approximately 50% of the lower explosive limit) and several orders of magnitude above anticipated human exposure levels.

There were no treatment-related effects in the parental animals, and no microscopic changes other than hydrocarbon droplet nephropathy in the kidneys of the male rats, which is known to be specific to the test animals and considered not relevant to humans. None of the reproductive parameters were affected, and there were no effects on offspring. The potential for endocrine modulation was also assessed by analysis of sperm count and quality as well as time to onset of developmental landmarks. No toxicologically important differences were found. Therefore, the reproductive No-Observed-Adverse-Effect-Level (NOAEL) in this study is 20 000 mg/m^3. No toxicologically important findings were identified by this study, and there is no need for product classification with respect to reproductive effects.

1. INTRODUCTION

Gasoline (CAS 86290-81-5) is one of the largest volume commercial products in the world with total consumption in Europe estimated at approximately 120 million metric tons/year [1]. From reviews of the toxicology data [2-5] it is apparent that gasoline has been extensively studied. However, some tests have been oral, some dermal, and others by inhalation, using either fully vapourized material or only the volatile fraction. There are also a number of studies of individual constituents or specific distillation cuts. Thus, it is not always clear how the results of these various tests relate to each other or how relevant they are to exposure conditions in occupational and consumer settings.

The toxicology data indicate that gasoline is not acutely toxic, except at very high concentrations [6-25] but can cause chemical pneumonitis if aspirated [26]. It may cause skin but not eye irritation, and is not sensitizing [6-8,12-14,16-18, 20,21,25,27-31]. In repeated dose toxicity studies, the most prominent finding is a specific change in the kidneys of male rats, subsequently shown to be an alpha-2µ-globulin-related process [32] which is both sex- and species-specific [33-38]. In carcinogenicity studies, exposure to fully volatilized gasoline resulted in the induction of kidney tumours in male rats, mediated by an alpha-2µ-globulin mechanism. It also resulted in liver tumours in female mice [39-41]. Gasoline is not mutagenic [25,42-67] and neither it, nor its blending components, have produced developmental toxicity [68-72]. One toxicological endpoint not previously addressed was reproductive toxicity. Thus, the objective of the present study was to investigate the potential of gasoline to cause reproductive toxicity.

The Technical Guidance Document for the European Union risk assessment process states that one approach is to "define the vapourized fraction representing actual exposure and conduct appropriate studies on this substance" [73].

Gasoline is a complex substance of variable composition, depending on the source of raw material (crude oil), refining processes, performance specifications, season, etc. Of particular importance is the fact that gasoline contains both volatile and non-volatile components, covering a wide distillation range. During gasoline production, storage, transportation and under normal conditions of consumer use the exposure is primarily to the volatile fraction via inhalation. Other routes of exposure, i.e. dermal and via ingestion, are restricted either to spillage, accidents or misuse and are not typical for everyday human exposure.

A number of studies have indicated that under occupational or environmental exposure conditions associated with routine production and use of gasoline, the composition of the vapour phase is quite different from that of the liquid gasoline [74]. In particular the low boiling components are present in the vapour phase in much higher proportions, whereas the high-boiling components are virtually absent.

For the reasons described above, it was decided to conduct a study of the reproductive toxicity of gasoline using the inhalation route and a sample of gasoline vapour rather than whole vapourized gasoline.

Within Europe, a number of gasoline distribution terminals have installed vapour recovery units (VRU). The condensed vapour from such an installation was assumed to represent the material to which workers and consumers may be exposed during

normal production and use of gasoline. Accordingly, a sample of VRU gasoline was collected and tested by inhalation for reproductive effects in order to provide appropriate data for gasoline reproductive hazard assessment and risk characterization. The study was a two-generation reproduction toxicity test in accordance with OECD Guideline 416 [75], and generally in agreement with other more recent recommendations in the United States (US) and Europe [76-78].

This report presents a brief overview of the project. A full account of the experimental conditions and detailed findings is presented elsewhere [79].

2. MATERIALS AND METHODS

2.1. MATERIALS

The test material used was vapour recovery unit gasoline, collected during 1995 from a distribution terminal in the Netherlands, where products were loaded into road tankers for onward distribution to retail service stations. This material is identified in the European Inventory of Existing Chemical Substances (EINECS) as vapour-recovery gasoline, CAS No. 68514-15-8. The specific vapour recovery technology in use at that terminal was from Kaldair® [80].

In the Kaldair® process vapours are first fed to a vapour holding tank and then to the VRU inlet. The composition of vapours fed to the VRU is expected to be reasonably constant over a 30 minute loading period because of the averaging effect within the holding tank. The VRU recovers hydrocarbons from the inlet stream by separating the volatile organic constituents from air in a pair of carbon adsorption beds. To obtain condensate, a desorption step is necessary. The constituents are then condensed to form a recovered product. The recovered product (condensate) runs down the condenser and into a receiving tank. When the tank is full, the recovered product is pumped back to storage. Thus, based on the characteristics of the VRU, it seemed likely that the recovered material would effectively comprise the volatile fraction of gasoline to which inhalation exposure occurs.

In order to verify the representative nature of the test material, samples of vapour from the terminal and condensed product were taken for chemical analysis. These analyses were then compared with vapour measurements at another terminal and to the results of gasoline exposure studies, particularly those relating to service station attendants and customers at self-service stations [74]. As the results of these analyses were all in reasonable agreement, arrangements were made to collect a large volume of VRU condensate for testing. A total of approximately 1760 kg was collected into eight storage vessels and shipped to the testing facility. Before use, material from each container was analyzed again to assure that the composition had not changed during shipment or storage. A summary of the analytical data is given in **Table 1**, with an analysis of a representative motor gasoline also provided for comparative purposes.

Table 1 Composition of Vapour and Bulk Gasoline

Component	Bulk Gasoline (1987)[1]	Service Station Self-Fill Exposure (1987)[1]	Bulk Gasoline (1996)[2]	Terminal Vapour (1995)[3]	VRU Test Sample (1995)[4]
Non-aromatics					
C_3 (% m)	0.1	3.4	--	0.3	1.0
C_4 (% m)	7.0	47.1	--	40.5	51.7
C_5 (% m)	19.1	26.2	--	42.4	37.2
C_6 (% m)	14.2	9.7	--	13.8	8.3
C_7 (% m)	7.9	1.7	--	1.5	0.4
C_8 (% m)	3.9	0.8	--	0.3	0.2
C_9 (% m)	0.9	0.1	--	--	--
C_{9+} (% m)	0.4	0.1	--	--	--
Total saturates (% vol.)	--	--	57.4	--	--
Total olefins (% vol.)	--	--	7.6	--	--
Aromatics					
Benzene (% m)	4.0	1.8	1.6	0.7	0.7
Toluene (% m)	12.2	1.6	--	0.5	0.7
C_8 (% m)	14.6	0.5	--	--	--
C_9 (% m)	9.2	0.2	--	--	--
C_{9+} (% m)	3.3	0.2	--	--	--
Total aromatics (% vol.)	--	--	35.0	--	--
Oxygenates & Unidentified	3.2	6.6	--	--	--

-- = No data available

1 CONCAWE gasoline exposure study [74]

2 Survey of 1996 Gasoline Quality in Europe [81]

3 Terminal measurement

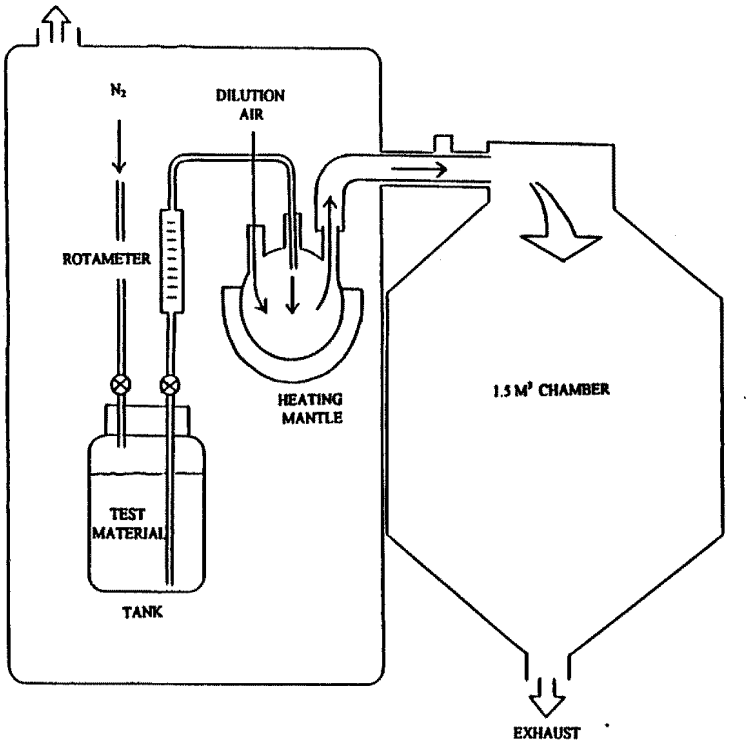
4 Final report for the validation study of the inhalation exposure system with vapour recovery unit condensate [82]

2.2. METHODS

Exposure Conditions

The animals were exposed six hours/day, seven days/week in approximately 1.5 m^3 chambers. A schematic of the test atmosphere generation and exposure system is presented in **Figure 1**. The temperature was maintained between 20-24^oC; the humidity ranged between 40-60%; and there were 12-15 air changes/hour. The target vapour concentrations were 5000, 10 000 and 20 000 mg/m^3. The chamber concentrations were monitored hourly via on-line gas chromatography (Hewlett Packard GC 6890).

Figure 1 Schematic of generation and exposure system



Experimental Design

A two generation reproduction toxicity study with Sprague-Dawley rats (CRL:CD®BR, obtained from Charles River Laboratories) was conducted via inhalation. The test was generally consistent with international guidelines for tests of this type [75-78] In addition the study was in compliance with US [83] and international [84,85] guidelines for good laboratory practice and with US guidelines for appropriate animal usage [86].

Briefly, groups of 30 randomly selected male and female Sprague-Dawley rats were exposed daily for six hours to test material at target concentrations of 5000, 10 000 and 20 000 mg/m^3. The highest concentration, approximately half of the lower explosive limit, was the highest level that was considered safe to test. The suitability of these levels was assessed in preliminary range finding studies [87-88] and, as no effects were observed, they were also used for the definitive study.

All animals were exposed for 10 weeks prior to mating and throughout the mating period (a maximum of three weeks). The males were sacrificed at this point; but exposure of the females continued until gestation day (GD) 20. Exposure was suspended until postpartum day (PPD) 5 to avoid undue stress to the dams while giving birth, and then restarted. Exposure of the females continued until sacrifice. Females confirmed to have mated by observation of a vaginal plug or the presence of sperm in a vaginal rinse were placed in single cages for the gestational period.

The pups were culled on a random basis to approximately 5/sex/litter. Culled pups were examined and sacrificed. At weaning on postnatal day (PND) 28, the pups (the first offspring generation, F_1) selected for the second generation were sorted by exposure group. Among the offspring not selected for mating, 3/sex/litter were sacrificed and examined for internal abnormalities. The remainder were examined externally, sacrificed and discarded. The selected pups were exposed for 13 weeks pre-mating and then for the three week mating period. The males were sacrificed at that point, and the females continued to be exposed until GD 20. Exposure was then resumed on PPD 5 and continued to weaning (PND 21) when all remaining animals were sacrificed.

Observations and Parameters Measured

All animals were checked twice daily for viability and clinical observations were carried out on a daily basis. Body weights and food consumption were measured weekly, until confirmation of mating and then on GD 0, 7, 14, and 21 and PPD 0, 4, 7, 14, and 21 for the first (P_1) and second (P_2) parental generation and on PPD 28 for P_1 only.

All pups were counted and examined externally on a daily basis until PND 21, and weighed on PND 0, 4, 7, 14, and 21. F_1 pups were also examined daily from PND 21 to 28 and weighed on PND 28 and 35.

All surviving F_1 and F_2 (second offspring generation) pups were evaluated for developmental landmarks including pinna detachment, hair growth, incisor eruption, eye opening, and the development of the righting reflex. Surviving F_1 female offspring were monitored for vaginal opening and F_1 male offspring were monitored for preputial separation.

Other parameters evaluated included: male and female fertility indices, male mating index, female fecundity and gestational indices, mean litter size, mean days of gestation, female oestrus cycle length and number of females cycling normally, total caudal epididymal sperm number, percent progressively motile sperm, total resistant spermatid count, percent morphologically normal sperm, percent of sperm with an identified abnormality, live birth index, survival indices (PPD 1, 4, 7, 14, 21), viability index at weaning, mean live and dead offspring at day 0, sex ratio at day 0, offspring in-life observations, offspring body weight, and offspring gross post-mortem findings.

Sacrifice, Necropsy, and Pathologic Examination

All animals dying spontaneously or sacrificed in a moribund condition were necropsied. Culled pups were examined externally but were not necropsied unless there was external evidence of abnormalities. Randomly selected pups were necropsied and the following organs weighed: ovaries, liver, adrenals, thymus, testes, kidneys, spleen and brain. Additionally the following tissues were taken for microscopic examination: vagina, ovaries, epididymides, prostate, pituitary, spleen,

kidneys, thymus, uterus (with cervix), testes, seminal vesicles, coagulating gland, adrenals, liver, brain, and any gross lesions.

Similar evaluations were carried out on all adults surviving to scheduled termination. Organs weighed included liver, adrenals, brain, uterus, testes, right epididymis and left caudal epididymis, seminal vesicles (with coagulating glands and fluid), kidneys, spleen, thymus, ovaries, prostate, and lungs. Tissues taken for microscopic examination included vagina, uterus, ovaries, right epididymis, seminal vesicles, prostate, oviducts, thymus, trachea, nasal turbinates, spleen, coagulating gland, pituitary, kidneys, liver, mammary gland (females only), testes, brain, larynx, lungs, adrenals, and any tissue masses/gross lesions. The tissues from the high dose and control animals were evaluated but as there was no evidence of treatment related effects, other dose groups were not evaluated.

Sperm samples were collected from all males for sperm count and morphology investigations. Ovarian examination included confirmation of growing follicles and corpora lutea and quantification of oocytes.

3. RESULTS

Exposure

The mean chamber concentrations (with standard deviations between brackets) of VRU gasoline during the study were 0 (0), 5076 (146), 10 247 (249), and 20 241 (373) mg/m^3.

Mortality, Weight Gain, and Clinical Signs

There were no treatment-related clinical signs of toxicity or mortality during the study. There were no significant differences in body weight gain or food consumption between treated and control animals of either generation. Similarly, there were no differences in gestational or postpartum body weights or food consumption in either generation.

Post-Mortem Observations and Organ Weights

There were no post-mortem findings that were considered unusual or appeared to be treatment-related. There were some isolated, statistically significant increases in absolute and relative organ weights. These were not considered to be treatment related, however, as there was lack of a dose-related response (i.e. the effects were not proportional to the exposure levels).

Pathological Investigation

There were no treatment-related microscopic changes in any of the reproductive tissues or in the tissues of the upper or lower respiratory tract from either of the parental (P_1 or P_2) generation rats exposed to 20 000 mg/m^3 VRU gasoline.

The only treatment-related changes observed were in male kidneys and consisted of an increase in the number and size of hyaline droplets present in both generations exposed, at all concentrations of VRU gasoline. These changes were consistent with the "hydrocarbon/hyaline droplet nephropathy" which is unique to male rats resulting from the exacerbated accumulation of alpha-2µ-globulin in the kidney [89].

Reproductive Parameters

No differences were observed between control and exposed parental animals of both generations for mating index, fecundity, pregnancy or length of gestation. Among the offspring of both generations there were no differences considered to be treatment related in mean litter size, fraction of live births, sex ratio, survival or body weight.

Sperm Parameters

No effects were observed on sperm count, motility, or gross appearance which were considered to be treatment related.

Oestrus Cycle

There were no statistically significant differences in mean oestrus cycle length, quantification of primordial oocytes, or percent females with abnormal cycles between treated and control females in the P_1 or P_2 generation.

Developmental Landmarks

There were no significant differences in incisor eruption, pinna detachment, or righting reflex in the F_1 or F_2 offspring, or vaginal patency or preputial separation in the F_1 offspring. There was a slight, but statistically significant delay in hair growth in the males but not females of the F_1 pups. Eye opening was advanced by approximately one-half a day for the high dose males and hair growth was delayed in the low dose males and females of the F_2 offspring. In the absence of a dose response relationship and/or effects in the high dose group, none of these findings were considered to be treatment related developmental effects.

4. DISCUSSION

The objective of this work was to assess the potential effects of gasoline exposure on reproductive processes. In this regard, the test substance, VRU gasoline, the volatile fraction of gasoline, was judged to be representative of the material to which humans are exposed. The study was carried out at levels up to 20 000 mg/m^3, approximately half of the lower explosive limit, and the highest level considered safe for use in the laboratory. This level is several orders of magnitude above occupational exposure measurements [74].

It was apparent that exposure of rats to VRU gasoline did not produce any pathological changes in reproductive organs. Additionally, there were no effects on mating, fertility, live births, birth weights, or survival and weight gain through weaning in two generations. Finally, there were no effects on sperm count, sperm quality, oestrus cycling, quantification of primordial oocytes, or developmental landmarks other than a slight delay in hair growth in some treated offspring. Thus, the reproductive NOAEL as defined by this study is equal to 20 000 mg/m^3.

Consistent with the findings summarized above, there were no effects in developmental toxicity studies of similar test substances at levels of approximately 25 000 mg/m^3 [69,72]. Thus there is now an extensive database of information which demonstrates that the volatile fraction of gasoline, to which humans may be exposed during normal handling, does not affect developmental or reproductive processes.

There are reports that chronic exposure of mice to fully vapourized gasoline reduces the incidence of enlarged/cystic uteri in mice [90]. It was suggested that this finding may be due to a reduction in oestrogen levels, as a consequence of enhanced liver metabolism in that sex and species. Although similar effects have not been observed in rats, the present study included assessment of several parameters which provide assurance that endocrine-mediated processes were unaffected by exposure to VRU. For example, there were no effects on fertility, sperm count, sperm quality, oestrus cycling, quantification of primordial oocytes, offspring sex ratio, body weight gain, pathological effects in target reproductive organs, and no changes in any specific landmarks related to sexual development in either generation. In summary, there were no effects of any kind that might have been a consequence of altered hormone balance.

With regard to systemic toxicity, the only effects observed were of a minor nature and were male-rat specific. There were no treatment-related effects on survival or weight gain. Some organ weights showed significant differences, but other than as described below, these were not dose-related, nor were they consistent between sexes or across generations.

The only consistent systemic findings related to kidney changes in the male rats. These changes included a slight increase in relative kidney weights in the high dose males from the second parental generation, as well as microscopic evidence of hyaline droplets in the male rat kidneys. However, as the weight difference was slight (less than 6%), and was restricted to males from one generation only, it was not considered to be adverse. The microscopic changes were consistent with an alpha-2µ-globulin-mediated process that is unique to male rats and not toxicologically relevant to humans [89,91].

On the basis of the results from this and other studies it is concluded that the volatile fraction of gasoline is not systemically toxic and does not affect reproductive parameters at levels up to at least 20 000 mg/m^3. Thus there is no apparent reproductive hazard associated with the substance and no basis for product classification.

5. CONCLUSION

In this study there were no toxic effects on the reproduction of rats exposed to gasoline vapour levels up to 20 000 milligram per cubic metre in air. The No-Observed-Adverse-Effect-Level for reproductive toxicity in rats for gasoline vapour derived from this study is equal to 20 000 milligram per cubic metre.

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