Regulatory Toxicology and Pharmacology 67 (2013) 446–455

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

The sub-chronic toxicity in rats of isoparaffinic solvents

Juan-Carlos Carrillo [a,*], M. David Adenuga [b], Richard H. McKee [b], Randy N. Roth [c], David Steup [d], Barry J. Simpson [e]

[a] Shell Health, Shell International B.V., P.O. Box 162, 2501 AN The Hague, The Netherlands
[b] ExxonMobil Biomedical Sciences Inc., 1545 Route 22 E., Annandale, NJ 08801, USA
[c] Roth Toxicology Consulting, P.O. Box 6023, Thousand Oaks, CA 91359, USA
[d] Shell Health, Shell Oil Company, P.O. Box 2463, Houston, TX 77252-2463, USA
[e] Simpson Toxicology Consulting, 4 Temple Farm Barns, Singledge Lane, Whitfield, Kent CT15 5AB, UK

ARTICLE INFO

Article history:
Received 31 May 2013
Available online 14 September 2013

Keywords:
Isoalkane
Isoparaffin
Sub-chronic toxicity
Inhalation toxicity
Occupational exposure limit
Liver enlargement
α2u-Globulin
UVCB
Hydrocarbon solvent

ABSTRACT

Results from a 13-week inhalation study in rats on a C10–C12 isoparaffinic solvent are compared to the results of repeated inhalation and oral toxicity studies of four other isoparaffinic hydrocarbon solvents. Statistically significant findings which were consistent across all studies included: nephropathy and small but significant changes in hematological parameters in male rats and liver enlargement in both male and female rats. The male rat kidney changes were due to an alpha 2u globulin process and not relevant for human health or risk assessment. The liver enlargement without pathologic changes or elevations in liver enzyme markers was considered to be an adaptive response. The reason for the reductions in hematological parameters that were observed in males only is not clear, but it is suggested that these were either due to normal variation or a secondary consequence of the nephropathy. The overall No Observed Adverse Effect Concentration (NOAEC) was the highest concentration tested in the study, >10,000 mg/m^3. Because of the overall pattern of response, this solvent is considered to be representative of low aromatic C9–C14 aliphatic solvents in general. The data are useful for risk assessment and other purposes including the development of occupational exposure recommendations.

1. Introduction

Isoparaffinic hydrocarbons are branched aliphatic molecules (iso-alkanes) that are used in a variety of applications from cosmetics, household cleaning products, paints, varnishes and in printing applications. The isoparaffinic substances covered in this study are complex solvents comprised of C8–C15 aliphatic hydrocarbon components and are members of the broader category of C9–C14 aliphatic solvents with low (<2%) aromatic content (also known as dearomatized mineral spirits[1]). Dearomatized mineral spirits and specifically isoparaffinic members of this broader category of aliphatic hydrocarbons show low order acute toxicity by the oral, inhalation and dermal routes of exposure (Amoruso et al., 2008; Mullin et al., 1990; Johnson et al., 2012). In repeated exposure studies, systemic effects of both dearomatized mineral spirits and isoparaffinic hydrocarbons follow similar toxicological patterns (Amoruso et al., 2008; Mullin et al., 1990; Johnson et al., 2012). On the basis of similarity in physical/chemical properties, carbon number range and overall toxicity profile, isoparaffinic solvents can be regarded as representative of the broader category of dearomatized C9–C14 aliphatic solvents.

Repeated exposure studies in rats (summarized in Amoruso et al. (2008)) provide evidence that the most commonly observed effect of these solvents are kidney changes in male rats. The earliest reports were in publications by Carpenter et al. (1975e,f, 1977b) who observed increased frequencies of moderate tubular degeneration in male rats in some repeated exposure studies and attributed this to an exacerbation of spontaneous, age-related renal lesions in male rats (i.e., chronic progressive nephropathy). It was noted that kidney changes were not observed in female rats or beagle dogs, establishing the species and gender specificity of these effects. These renal changes were further characterized in studies of isoparaffinic hydrocarbons by Phillips and Egan (1984a,b) who described the gross and clinical changes associated with repeated exposure to these solvents and by Phillips and Cockerell (1984) who characterized the findings histologically. Phillips and Cockerell also drew attention to similar observations by Alden and Stone (later published as Alden et al. (1984)) in studies of decalin, a cycloparaffinic hydrocarbon, in which the effects were associated with the induction of a specific urinary protein (α2u-globulin). In these early papers, the effects were characterized as light hydrocarbon nephropathy, because of the substances that caused it, or hyaline droplet nephropathy, because of the

* Corresponding author. Tel.: +31 703772576; Fax: +31 703772840.
E-mail address: juan-carlos.carrillo@shell.com (J.-C. Carrillo).

[1] Dearomatized mineral spirits are aliphatic hydrocarbons which meet the specifications of ASTM D-235 (1995).

0273-2300/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yrtph.2013.09.004

characteristic histological findings. Evidence of similar findings in studies of motor gasoline (MacFarland et al., 1984) led to studies to identify the most active constituents which ultimately associated these effects with isoparaffinic structures (Halder et al., 1985; Aranyi et al., 1986) including 2,2,4-trimethylpentane which became a model compound for mechanistic studies (Short et al., 1986; Cuervo et al., 1999). It is now known that these renal effects in male rats are the consequence of a process by which substances of diverse structure bind to α2u-globulin. The complexes are reabsorbed in the proximal tubules where they accumulate in lysosomes in renal epithelial cells. This leads to a cytotoxic and regenerative process which if sustained for at least a year can ultimately lead to the development of renal cell carcinoma (Swenberg et al., 1989). However, as this process has an absolute requirement for α2u-globulin which is most commonly found in male rats but not in other species including humans, these effects and their consequences are regarded as male rat specific and not relevant to human health (US EPA, 1991; Swenberg and Lehman-McKeeman, 1998; Hard et al., 2013).

Other effects related to exposure to isoparaffinic solvents included hematological or urine chemistry changes, reported in several studies. These were not considered toxicologically relevant or considered secondary to other effects because they lacked a dose–response pattern, were mostly minor changes and not consistent across gender (Phillips and Egan, 1984b; Schreiner et al., 1998). Rector et al. (1966) compared the effects of a C9–C14 aliphatic solvent (13–19% aromatics) in rats, rabbits, dogs, monkeys and guinea pigs at exposure levels up to approximately 12,000 mg/m^3. There were no notable effects on animals of any species other than guinea pigs, and, as shown in subsequent studies by Jenkins et al. (1971), the effects in guinea pigs were exacerbated by the consumption of a diet low in vitamin C. When dietary levels of vitamin C were sufficient, guinea pigs were much less susceptible to the effects of the solvent.

Subsequently, a series of inhalation toxicity studies on C9–C14 aliphatic hydrocarbons was published (Carpenter et al., 1975a–g; Carpenter et al., 1976a–e; Carpenter et al., 1977a–c; Carpenter et al., 1978; Nau et al., 1966). As summarized by Amoruso et al. (2008), no consistent treatment-related effects were reported other than kidney effects in male rats and some minor changes in hematologic parameters. A summary of studies available on isoparaffinic solvents with carbon numbers ranging from C10 to C15 was published approximately 20 years ago (Mullin et al., 1990), and more recently, a safety assessment of isoparaffins as used in cosmetics was published (Johnson et al., 2012). However, the former publication did not include all relevant data including the results of the study reported herein, and the latter was specifically aimed at cosmetic usage. In the studies reviewed by Mullin et al. (1990) and Johnson et al. (2012), there were no significant systemic effects other than mild to moderate kidney lesions in male rats, mild increases in relative and absolute liver weights and minor hematological changes in male rats.

The purpose of the present publication is to report on a 13-week inhalation study that has been carried out in rats on a C10–C12 isoparaffinic solvent and to compare and contrast the results with those from repeated exposure studies in rats using similar solvents (Phillips and Egan 1984a,b; Schreiner et al., 1998). A further objective was to assess the extent to which the conclusions for these studies could be applied to the broader group of C9–C14 aliphatic hydrocarbon solvents for purposes of hazard characterization and/or in the development of occupational exposure limits for establishing risk management measures.

2. Materials and methods

2.1. Test material

The toxicological data described in detail in this paper were based on an inhalation toxicity study of a high-boiling aliphatic hydrocarbon solvent consisting of (>98%) isoparaffins, predominantly in the carbon number range C10–C12 (CAS number 64741-65-7, conventionally named as "hydrocarbons, C10–C12 isoalkanes, <2% aromatics"). The physical/chemical properties and compositional analysis of the sample are shown in Tables 1 and 2.

2.2. Experimental procedure

The animal experimentation was carried out following procedures equivalent to those of Organization for Economic Cooperation and Development (OECD) 413. Briefly, aluminum exposure chambers (1 m^3) were used; these were ventilated by air drawn from the laboratory through dust filters. The exhaust ducts from each chamber entered a common exhaust duct through which the air was drawn by fan at a rate of 2.0 ± 0.03 m^3 per minute. Air flow rate was monitored continuously throughout the experiment using an electro-anemometer mounted in the duct and slight adjustments made when necessary to maintain a constant exhaust rate. Individual flow rates through each chamber were balanced and adjusted to 0.50 m^3 per minute before exposures began, but were not checked during the exposure.

Test atmospheres were generated by complete volatilization of test material into the stream of ventilating air. The vaporizers were electrically heated quartz tubes whose surface temperatures were adjusted during preliminary experiments to the minimal required for complete vaporization of the test material. A total hydrocarbon analyzer fitted with a flame-ionization detector (Beckman 109A) was used to sequentially analyze the test atmospheres.

Thirteen week old Specific Pathogen Free (SPF) Wistar rats were housed in groups of three by sex, in hanging aluminum cages fitted with steel mesh bases. Underneath the mesh bases, paper-lined catch trays were placed to catch excreta; the trays were changed daily. There were twelve cages arranged in two layers in each

Table 1
Physical chemical properties of the C10–C12 isoparaffinic solvent.

Property/parameter	Value
Relative density (15.6/15.6 °C)	0.750
Distillation range	170–187 °C
Flash point (Abel)	46 °C
Flash point (TAG)	51 °C
Color Saybolt	+30
Total sulfur content	<0.001% (w/w)
Copper corrosion	No. 1 strip
Aromatics content	<0.5% (v/v)
Aniline point	84 °C
Kauri-Butanol value	26
Relative evaporation rate (*n*-butyl acetate = 1)	<0.1

Table 2
Composition of the C10–C12 isoparaffinic solvent by hydrocarbon type.

Carbon number and type	Content (% w/w)
C8 Paraffins	<1
C9 Paraffins	1
C9 Naphthenes	<1
C10 Paraffins	15
C10 Naphthenes	1
C11 Paraffins	39
C12 Paraffins	44
Total paraffins	99
Total naphthenes	1