The Use of Information on Upper Respiratory Tract Irritation and Acute Central Nervous System Depression to Set Occupational Exposure Limits for Hydrocarbon Solvents

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ABSTRACT

Hydrocarbon solvents are liquid hydrocarbon fractions, often with complex compositions. Because of the potential for human exposure, primarily to the more volatile solvents, considerable effort has been directed towards the development of occupational exposure recommendations. Because of the complex and variable nature of these substances, the recommended approach is to calculate occupational exposure levels (OELs) using an adaptation of the mixture formula developed by the ACGIH® in which "group guidance values" are assigned to similar constituents. This approach is supported by the results of toxicological studies of hydrocarbon solvents and their constituents which have shown that, with a few, well characterized exceptions, exposures to these substances do not cause toxicologically important systemic effects. Accordingly, the effects that seem most appropriate for use in setting OELs are upper respiratory tract irritation and acute central nervous system (CNS) depression. In early studies, volunteers were exposed to these substances over a range of concentrations to identify exposure levels suitable for occupational settings. In more recent years, for ethical reasons, there has been a shift to the use of animal data to characterize these effects, particularly the potential for acute CNS effects, with volunteer studies now being used primarily to confirm that the recommended exposure levels are below effect levels. Using the data from animal studies, along with physiologically-based pharmacokinetic (PBPK models), it is possible to predict no effect levels in humans, and to then compare the predictions to empirical evidence from human studies. These comparisons provide evidence that the recommended approach to setting OELs for complex hydrocarbon solvents yields recommendations that are below the no effect levels for acute effects while also providing protection from longer term effects associated with the more problematic constituents.

INTRODUCTION

Hydrocarbon solvents are liquid hydrocarbon fractions derived from crude oil, intended specifically for solvent use.[1] These solvents collectively span a carbon number range of approximately C5-C20 and may contain normal-, branched (iso-), cyclic (cyclo-) aliphatic constituents and/or aromatic rings. Although some hydrocarbon solvents are mono-constituent (e.g., n-pentane), many are complex and may contain large numbers of isomers of some or all of the 4 hydrocarbon types. Because some hydrocarbon solvents are volatile, it is important to provide occupational exposure recommendations to allow users to control hazards in specific work environments. Indeed this has been an area of active investigation for many years. One specific difficulty is that many hydrocarbon solvents have complex and variable compositions, due in part to the methods of production and the technical requirements. Additionally, with increasing molecular weight the numbers of isomers becomes very large, and it is difficult to identify all possible constituents, let alone characterize their hazards individually. However, with a few, well characterized exceptions, hydrocarbon solvent constituents have very similar toxicological properties and can be assessed on a generic basis.

Historically the toxicological properties of hydrocarbon solvents have been characterized via a representative substance approach, and the results have then been verified with studies of specific constituents (for further discussion on this subject see McKee et al., 2015). In a systematic assessment of the toxicological hazards of volatile hydrocarbon solvents and related materials, Carpenter et al. (1975a-h, 1976a-e, 1977a-c, 1978) tested 16 commercial products for acute effects in rats and mice, repeated dose effects in rats and beagle dogs, and also exposed volunteers for short periods of time to assess the potential for irritation and acute CNS effects. One objective of the Carpenter studies was to provide base data that could be used to set occupational exposure limits. One outcome of the Carpenter studies was the observation that hydrocarbon solvents produce minimal systemic effects; more specifically, the only effects, observed in some studies, were pathological changes in the kidneys of male rats that were believed at the time to be an exacerbation of chronic progressive nephrosis but are now understood to be the consequence of an α2u-globulin-mediated process that is sex and species dependent and not relevant to humans (USEPA, 1991; Swenberg and Lehman-McKeeman, 1998).[2] From this work Carpenter drew three over-arching conclusions: (1) that the effects of hydrocarbon solvents most relevant to occupational exposure recommendations were upper respiratory tract irritation and acute central nervous system (CNS) depression; (2) that the aromatic and cycloparaffinic constituents were more irritating than the corresponding alkanes; and (3) that these properties were additive (Carpenter et al., 1977c).

More recent repeated exposure studies have provided additional evidence that most hydrocarbon solvents and their constituents do not produce toxicologically relevant

[1] A hydrocarbon solvent is defined as a chemical compound composed of carbon and hydrogen and capable of dissolving another substance (Lewis, 1993). In this context hydrocarbon solvents differ from petroleum-derived fuels which have a common origin and similar processing steps but have wider boiling ranges and may have higher levels of problematic constituents.

[2] Note that Carpenter hypothesized that these effects were an exacerbation of chronic progressive nephropathy, a spontaneous aging lesion. In subsequent studies it was reported that these effects are caused by isoparaffinic hydrocarbons and, in the early literature, referred to as “light hydrocarbon nephropathy”.

systemic effects. As discussed in detail in reports summarizing information on key studies (Adenuga et al. 2014a; 2014b; Carrillo et al., 2013; 2014), the common systemic effects associated with repeated exposure to representative solvents of different types are α2u-globulin-mediated kidney changes in male rats, increased liver weights, and, in a few studies, small but statistically significant reductions in hematocrit and/or hemoglobin content. The renal effects are species-specific and not relevant to humans (US EPA, 1991; Swenberg and Lehman-McKeeman, 1998); the increased liver weights are an adaptive response to increased metabolic demands (Maranpot et al., 2010); and, although the underlying mechanism for the hematological changes is unknown, the differences are within normal physiological limits and not considered toxicologically important (Car et al., 2006). The only known exceptions to these generalizations are n-hexane, the specific isomers of diethyl- and triethylbenzene which have ethyl groups are on adjacent carbons, and naphthalene. n-Hexane produces a peripheral neuropathy through a process involving the formation of a γ-diketone metabolite (2,5-hexanedione) (IPCS, 1991). Whereas other structurally similar substances including n-pentane, other hexane isomers, cyclohexane and heptane are not metabolized to a γ-diketone at toxicologically relevant levels and do not produce chronic neurological effects (Egan et al., 1980; Frontali et al., 1981; Ono et al., 1981; Takeuchi et al., 1980). The diethyl- and triethylbenzenes with the ethyl groups on adjacent carbons also produce a neurotoxic effect that is similar to the effect of n-hexane through a process for which the mode of action requires the formation of specific acyl metabolites (Kim et al., 2001 2002, Sabri et al., 2007). The other diethyl- and triethylbenzene isomers cannot form this metabolite and are not neurotoxic (Gagnaire et al., 1990, 1991, 1992, 1993). Naphthalene is highly irritating and has caused respiratory tract tumors in chronic studies in rodents. It is believed that the effects of naphthalene are related to metabolites formed by ring oxidation (Morris and Buckpitt, 2009). This distinguishes naphthalene from most of the other aromatic constituents of hydrocarbon solvents which are primarily metabolized by side chain addition. It should be noted, however, that ring oxidation is one of the pathways (although minor, depending on length of alkyl chain) by which alkylnaphthalenes are metabolized, and like naphthalene, these molecules can also be quite irritating, at least in rodents.

The problem of setting occupational exposure limits for complex hydrocarbons thus becomes a question of how to avoid vapor concentrations that are irritating or cause acute CNS effects while also assuring that the occupational exposure recommendations for specific constituents are not exceeded. An approach to dealing with the compositional variability was proposed by McKee et al. (2005), by which occupational exposure levels were calculated for hydrocarbon solvents through the use of a reciprocal calculation formula[3] previously developed by the ACGIH® for mixtures. In the calculation, "guidance values" were used to represent ranges of hydrocarbon solvent constituents with similar physical and chemical properties, and occupational exposure limits were then calculated using the relationship:

$$F_a/OEL_a + F_b/OEL_b + F_c/OEL_c + \ldots = 1/OEL_{\text{complex hydrocarbon solvent}}$$

In which F_a is the mass fraction of constituent a, OEL_a is the exposure limit for constituent a, F_b is the mass fraction of constituent b and OEL_b is the exposure limit for constituent b, etc.

[3] This is the Reciprocal Calculation Procedure (RCP) by which an occupational exposure limit can be calculated for a complex hydrocarbon solvent based on its composition.

These recommendations were incorporated into Appendix H of the ACGIH® guidance (ACGIH, 2015) (Table 1).

Table 1. Group Guidance Values (GGVs) and ACGIH TLV® values for specific hydrocarbon solvent constituents (Table adapted from Appendix H of ACGIH TLV® documentation).

Hydrocarbon Solvent Constituent Group	Group Guidance Value/Specific Substance Value	Constituents with ACGIH TLVs®
C5-C8 Alkanes	1500 mg/m^3	Pentane, isomers – 2950 mg/m^3 Hexane isomers – 1760 mg/m^3 Heptane isomers – 1640 mg/m^3 Octane isomers – 1400 mg/m^3 Cyclopentane – 1720 mg/m^3 Cyclohexane – 350 mg/m^3
C9-C15 alkanes	1200 mg/m^3	Nonane – 1050 mg/m^3
C7-C8 aromatics [1]	200 mg/m^3	Toluene – 75 mg/m^3 Xylene, all isomers – 434 mg/m^3 Ethylbenzene – 87 mg/m^3
C9-C15 aromatics	100 mg/m^3	Trimethylbenzene isomers – 100 mg/m^3
Naphthalene	50 mg/m^3	50 mg/m^3
n-Hexane	175 mg/m^3	175 mg/m^3

[1]Since these values were proposed there have been reductions in the ACGIH TLV® values for toluene and ethylbenzene. As a result, it has been recommended that this guidance value be withdrawn in favor of the TLV® values for the specific substances when they are present in solvents at levels high enough to impact the overall calculation.

One objective of the guidance was to provide reasonable assurance that the occupational exposure recommendations for specific hydrocarbon solvent constituents would not be exceeded as long as the calculated occupational exposure limit for the solvent was respected. Accordingly, the development of guidance values was strongly influenced by consensus occupational exposure recommendations for hydrocarbon solvent constituents as they existed at the time the method was developed. In particular the recommendations were influenced by the ACGIH TLV® values and by the recommendations of the European Scientific Committee for Occupational Exposure Limits (SCOEL). More recently, detailed characterizations of the hazards (physical/chemical, toxicological, environmental) of hydrocarbon solvents were prepared to meet the needs of the Organization for Economic Cooperation and Development (OECD) High Production Volume (HPV) initiative and to register hydrocarbon solvents in accordance with the European REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) Regulation. These recent data

compilations afford the opportunity to assess whether the underlying assumptions of the RCP are supported by the underlying toxicological information.

Historically, the potential for irritation and acute CNS effects of hydrocarbon solvents has been assessed in volunteer studies such as the previously mentioned Carpenter studies. Early studies encompassed a range of exposure levels to establish no effect levels; however, human studies are now more commonly used to confirm that occupational recommendations are appropriate and exposures do not normally exceed occupational levels. Although irritation can probably be best assessed in humans, acute CNS effects are now most commonly investigated in animal studies. Because of the presumed relevance of acute CNS effects to occupational exposure levels for volatile hydrocarbon solvents, a series of studies was conducted to define a battery of tests by which similar effects could be assessed in humans and animals (Hissink et al., 2007; 2009; Lammers et al., 2007; 2009; 2011; McKee et al., 2006; 2010; 2011). As the studies have been completed and published, it is appropriate to review the information to determine whether protection against acute CNS effects is afforded by the occupational exposure recommendations for this group of substances.

ACUTE CENTRAL NERVOUS SYSTEM DEPRESSION

Acute CNS depression is a condition of diminished mental function due to reductions in release of neurotransmitters. With respect to hydrocarbon solvents acute CNS depression is related to the levels of hydrocarbons in the central nervous system and has, as its most sensitive indicators, disinhibition and reduced attention. However, at exposure levels that are sufficiently high, the effects can become more profound, eventually leading to loss of consciousness or even death. Indeed, some of the more volatile hydrocarbon solvent constituents were investigated as potential anesthetic agents (e.g., Virtue, 1948). Normally acute CNS effects are quickly reversed when exposures are terminated, and no long term consequences are known unless the depression had been so profound as to cause anoxic hypoxia. Some of the earliest occupational exposure recommendations for hydrocarbon solvents were intended to avoid the onset of overt acute effects including acute central nervous system (CNS) depression, which could impact job performance and/or limit the ability to escape emergency situations, as well as ocular and upper respiratory tract irritation which could produce intolerable conditions in the workplace.

Experimental Studies to characterize the potential for hydrocarbon solvents to produce acute CNS effects in humans and experimental animals

To characterize the acute CNS effects of hydrocarbon solvents, an experimental program was initiated to identify a battery of tests that could be used in rats and humans. Studies were then conducted using these tests in both rats and volunteers to obtain observational information for two model substances, white spirit[4] and cyclohexane, and to also collect

[4] "White spirit" is a trade name for a hydrocarbon solvent containing approximately 80% C9-C11 aliphatic constituents and 20% aromatic constituents, primarily isomers of trimethylbenzene and ethyltoluene. The solvent was invented in 1927 for use in the dry cleaning industry with technical specifications which, among other things, limited the aromatic content to a maximum of 25%. This

information that could be used in developing physiologically-based pharmacokinetic models to relate the human and animal data. Finally, the acute CNS effects of a number of hydrocarbon solvent constituents and representative solvents were assessed in studies in rats to develop a method to predict the acute CNS effects of hydrocarbon solvents based on compositional information. The overall objective was to compare the predicted values to occupational exposure recommendations and human observations to assure that acute CNS effects would not occur when the recommendations were observed.

The first of these studies (McKee et al. 2006) used ethanol as a reference substance to characterize tests of acute CNS effects in rats and humans. Ethanol was administered to volunteers at levels sufficient to achieve blood levels approximating the legal limits for motor vehicle operation in the Netherlands (the country in which the testing was performed). A battery of functional and neurobehavioral tests was administered, and blood level data was obtained. As expected, some functional impairment was observed after dose administration, but this resolved over a period of hours as the alcohol was metabolized and eliminated. The testing in animals was analogous, but the administered doses were substantially higher. The results were qualitatively similar in that similar domains were affected in both species, but there were qualitative differences related to species differences in ethanol metabolism.

Studies of "white spirit" and cyclohexane were then conducted in both rats and volunteers, using this test battery to obtain observational data as well as information that could be used in the development of pharmacokinetic models (Lammers et al., 2007; 2009). Volunteers were exposed for 4 hours to "low" and "high" levels of the selected substances with the highest levels being 570 mg/m^3 for "white spirit" and 860 mg/m^3 for cyclohexane, approximating the occupational exposure limits in the Netherlands. The volunteers were tested prior to, during, and 90 minutes after the exposure period. Blood samples, taken 10 minutes prior to the end of the exposure period, were considered to reflect steady-state conditions. The rats were exposed at higher levels, 8 hours/day for 3 consecutive days and tested for neurobehavioral effects immediately after being removed from the exposure chambers on each of the treatment days and then 24 hours after the final exposure. Separate groups of animals were sacrificed at regular intervals to collect blood and tissue to measure hydrocarbon levels in these compartments. Because white spirit is a complex solvent, two marker compounds, n-decane and 1,2,4-trimethylbenzene (TMB) were selected to represent the aliphatic and aromatic fractions. In the white spirit studies, the principal effect in rats was an increase in time to respond in a performance test in which the rats had been trained to press a lever in response to a visual cue (Lammers et al., 2007). Statistically significant differences were found among rats exposed to either 2400 mg/m^3 or 4800 mg/m^3; the no effect concentration was 600 mg/m^3. There were no effects in rats tested 24 hours after exposure, confirming the acute and reversible nature of these findings. Among the volunteers exposed to 570 mg/m^3, the finding most closely linked to exposure was an approximately 5% increase in time to respond in a simple reaction time test. Although the difference was statistically significant, the effects were too subtle to be noticed by the

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product was originally named Stoddard (or Stoddard's) solvent after its inventor, but has traditionally been called "white spirit" in Europe and "mineral spirit" in the United States. The sample used in the studies by Hissink et al. (2007) and Lammers et al. (2007) contained 25.6% aromatics with the remainder being C9-C12 aliphatic constituents.

subjects and most likely within the normal range (Blake, 1971). The cyclohexane studies (Lammers et al., 2009) followed a similar design. In rats exposed at levels up to 28000 mg/m^3, 8 hours/day for 3 consecutive days, there was some limited evidence of tremor and effects on gait, sensorimotor reactivity and psychomotor speed in the high exposure group, but all of the differences were small and their relationship to treatment was uncertain. The no effect concentration was 8000 mg/m^3. In the volunteer studies, there were no objective findings in subjects exposed to 860 mg/m^3 for 4 hours, but there was an increase in some subjective complaints. The possibility that the subjective findings were influenced by odor must be considered.

The blood and tissue levels were used in PBPK models to compare no effect levels in humans and rodents. More specifically, Hissink et al. (2007; 2009) estimated the brain concentrations in humans corresponding to those measured in the rat studies at equivalent external exposure levels. In the white spirit study, at 600 mg/m^3, the no effect level in rats, the constituent concentrations in the central nervous system were 1175 ng/g n-decane and 430 ng/g TMB. The predicted concentrations in the human central nervous system at equivalent external exposure levels were 950 ng/g n-decane and 721 ng/g TMB (Hissink et al., 2007). In the cyclohexane study, the no effect concentration in rats (8000 mg/m^3) corresponded to a predicted human exposure level of 4200 mg/m^3. Thus, assuming the degree of acute CNS effects in humans and rats to be similar at similar central nervous system concentrations, the no effect levels for linear and branched alkanes in humans would be similar to those in rats, whereas for cycloparaffins and aromatics, the human no effect levels would be approximately half the corresponding no effect levels in rats.

The acute CNS effects of hydrocarbon solvent constituents over the range of C5-C10 were then characterized in rats. Higher molecular weight constituents (i.e., C10-C20) were not tested as the vapor pressures are too low to allow the generation of concentrations high enough to produce effects. Studies by Nilsen et al. (1988), and, later Bowen and Balster (1998) provided empirical evidence that the highest sustainable concentrations of C10+ n- and iso-paraffins do not produce acute CNS effects in rodents.

The C5 constituents (n-, iso-, cyclopentane) did not produce acute CNS effects at 20,000 mg/m^3, the highest exposure levels tested (approximately half of the lower explosive limit) (Lammers et al., 2011; McKee et al., 2010; 2011). The absence of effects at these levels is consistent with evidence that C5 aliphatic constituents are poorly absorbed and rapidly eliminated (see McKee et al., 1998 for more information). Indeed, Virtue (1948) reported that cyclopentane could produce profound CNS depression leading to respiratory arrest and death in rats but only at levels $\geq$ 8% (i.e., approximately 80,000 ppm, or 240000 mg/m^3).

Acute central CNS effects were not observed in studies of n- and iso-octane at exposure levels of 14,000 mg/m^3, the highest concentrations tested (Lammers et al., 2011; McKee et al., 2011). A complex C6-C7 cycloparaffinic solvent produced minor reversible effects at 14,000 mg/m^3 with a no effect concentration of 4200 mg/m^3 (McKee et al., 2011).

n-Decane and a complex C9-C11 isoparaffinic solvent produced minor, reversible CNS effects at 5000 mg/m^3 with 1500 mg/m^3 as the no effect level; whereas a C10 cycloparaffinic solvent did not produce acute CNS effects at 5000 mg/m^3, the highest concentration tested (Lammers et al., 2011; McKee et al. 2011).

In studies of C9-C11 aromatics (alkylated benzenes and naphthalenes), no effect levels ranged from approximately 200 mg/m^3 to 1250 mg/m^3 (McKee et al., 2010).

Other investigators have reported similar results. Christoph et al. (2000) reported that there was no evidence of acute CNS effects in rats exposed for 6 hours to cyclohexane at levels up to 2000 ppm (6000 mg/m^3) but that small effects were observed in the 7000 ppm (21000 mg/m^3) exposure group. Boyes et al. (2000) reported that inhalation exposure of rats to 1,2,4-trimethylpentane (an iso-octane isomer) altered visual evoked potentials and signal detection behavior after 62 minutes of exposure at 2500 ppm (approximately 12000 mg/m^3) with 2000 ppm (9500 mg/m^3) as the no effect concentration. Korsak and Rydzynski (1996) reported that in acute exposure studies of each of the 3 trimethylbenzene isomers, acute effects were observed a levels > 2000 mg/m^3. Cushman et al. (1995) reported that exposure to cumene at levels $\geq$ 2500 mg/m^3 increased motor activity. Douglas et al. (1993) showed that repeated exposure to a mixed C9 isomer aromatic solvent had no persistent effects on the central nervous system. Thus, the data from Lammers et al. (2006; 2009; 2011) and McKee et al. (2010; 2011) are consistent with results from other investigators but most suited for an overall comparison as they represent the largest data set obtained using a common protocol.

Hydrocarbon Solvent Constituent Distribution to the Central Nervous System

The evidence of acute CNS effects summarized above paralleled the kinetic data from Zahlsen et al. (1992; 1993) who showed that the concentrations of n- and iso-alkanes in the central nervous system increase with increasing carbon number from C6 to C10 (Figures 1a-c). This is consistent with the evidence that the lowest no effect levels (1500 mg/m^3) for the alkanes were achieved with n- and iso-decane. The central nervous system concentrations of cycloparaffins were higher than those of the corresponding n- and iso-alkanes for constituents with carbon numbers $\leq$ C8, but the levels then decreased as the carbon numbers increased. The finding that the highest concentrations of cycloparaffins were found for C8 constituents is consistent with the experimental evidence that lower no effect levels for acute CNS effects were found for the C6 and C7 cycloparaffinic constituents than the C10 cycloparaffins. As shown by Nilsen et al. (1988), it is not possible to achieve stable vapor concentrations at concentrations of C10+ n-alkanes to produce acute CNS effects. The authors attributed this in part to the limited saturated vapor concentrations imposed by the lower vapor pressures of the higher molecular weight constituents, but it was also noted that the blood/air and brain/air ratios declined for n-alkanes with carbon numbers > C10, indicating that these higher molecular weight constituents were not as efficiently distributed to the brain as is n-nonane. Hau et al. (2001) suggested that blood/brain barrier effects may inhibit uptake of higher molecular weight alkanes.

Considering the data above, across the range of aliphatic hydrocarbon solvent constituents, 1500 mg/m^3, the no effect level for the C10 n- and iso-alkanes, was the lowest value reported. Based on PBPK modeling, the predicted no effect level for n-alkanes in humans approximates that experimentally defined in rats (Hissink et al., 2007). Thus, the human no effect level for acute CNS effects of C5-C10 alkanes should not be lower than the no effect concentration for n-decane, 1500 mg/m^3. Based on the data for cycloparaffinic hydrocarbons, the lowest no effect level of 4200 mg/m^3 for a complex C6-C7 would approximate the lowest no effect concentration for these constituents. The theoretical

analysis from Hissink et al. (2009) predicted that the human no effect levels would be approximately half the levels measured in rats, making the lowest predicted no effect level in humans approximately 2100 mg/m^3.

The kinetic data for aromatics does not provide an upper concentration limit as the highest measured value was recorded for a C10 aromatic which was also the highest molecular weight species tested. However, as the low vapor pressures for these constituents limit the potential exposures to higher molecular weight constituents, it would be reasonable to assume that the lowest no effect level would approximate the lowest value obtained from the rodent rodents (200 mg/m^3, McKee et al., 2010). The theoretical analysis predicted that no effect levels in humans would be approximately half the levels in rats (Hissink et al., 2006), making the overall lowest no effect level in humans approximately 100 mg/m^3.

HUMAN EVIDENCE

Historically occupational exposure recommendations were based on observations of workers and tested in studies in which volunteers were exposed for short periods of time to levels high enough to establish no effect levels for upper respiratory tract irritation and/or acute CNS depression. Among the earliest of the volunteer studies was a report by Patty and Yant (1929) on the CNS effects of volatile aliphatic hydrocarbons. Nelson et al. (1943) conducted human investigations of the effects of Stoddard solvent along with other industrial solvents. Nau et al. (1966) used volunteer testing as the basis for their occupational exposure recommendations for alkylbenzene-containing solvents. And Carpenter and associates (1975a-h, 1976a-e, 1977a-c, 1978) used volunteer studies to either provide occupational exposure recommendations for commercial hydrocarbon solvents or to confirm that the advice being provided by the ACGIH® was reasonable. Although many of the hydrocarbon solvents tested by Carpenter and associates are no longer commercially available, some are still in use, and the data are relevant to the determination of no effect and low effect levels. In other cases, the solvents have been replaced, and, although the data are not directly relevant, they may still be useful for comparative purposes, it may not be directly relevant to the present situation. One aspect of these studies is that the volunteers were exposed over a range of concentrations including levels that produced overt effects as a means of identifying tolerable workplace conditions. However, since the development of ethical standards for human testing (e.g., World Medical Association, 1989), volunteer studies have normally been conducted to validate occupational exposure recommendations derived from animal data, and human exposures are restricted to levels that are not expected to cause undue discomfort or other adverse effects.

Aliphatic Solvents

The more limited human data for complex aliphatic solvents is related to time at which these substances were introduced into commerce. More specifically, although some normal paraffinic solvents have been available at least since the early part of the 20th century, the first complex hydrocarbon solvent, Stoddard solvent (also known as white spirit or mineral spirit), a solvent containing approximately 80% aliphatic and 20% aromatic constituents, was introduced in the 1920s. Less volatile, low aromatic solvents became commercially available in the US in the 1960s and in Europe in the early 1980s, and, since their introduction, these

solvents have replaced comparable products with higher aromatic levels in many commercial applications. In recent years, most of the human studies have been of either aromatic solvents (as described in the previous section) or higher molecular weight aliphatic solvents, in particular comparisons of the complex aliphatic/aromatic solvents to the comparable low aromatic grades.

In one of the earliest volunteer studies with normal paraffinic hydrocarbons, Patty and Yant (1929) exposed volunteers in groups of 3 to 6 to vapors of aliphatic hydrocarbons with 4 to 7 carbons. They reported that exposure to n-pentane at levels up to 0.5% (approximately 15000 mg/m^3) did not produce CNS effects: that exposure to hexane at 0.2% (approximately 7000 mg/m^3) did not produce CNS effects but that there was some evidence of acute CNS effects at 0.5% (approximately 17,500 mg/m^3); and that among humans exposed to n-heptane there was slight evidence of acute CNS effects at levels as low as 0.1% (approximately 4000 mg/m^3), the lowest concentration tested.

Complex aliphatic solvents with low aromatic content were not widely available commercially when the Carpenter studies were being conducted, so the majority of the studies of low aromatic solvents have primarily been confirmatory rather than investigative studies, primarily comparisons of low aromatic hydrocarbon solvents to "aliphatic/aromatic" solvents such as "white spirit" in which aromatics may comprise as much as 25% of the total volume of the solvents. In an early publication, Pedersen and Cohr (1984) compared the responses of 12 volunteers to exposure for 6 hours at approximately 600 mg/m^3 to a C10-C12 isoparaffinic solvent (in which the levels of aromatics are very low; <2%), a complex C9-C11 solvent which has been processed (de-aromatized) to reduce the concentration of aromatics to low levels (<2%), and regular "white spirit" containing 18% aromatics. This study was followed by a second in which the volunteers were exposed to the de-aromatized solvent for 6 hours at levels of approximately 300, 600, or 1200 mg/m^3. Pedersen and Cohr (1984) reported that none of the volunteers experienced any signs or symptoms of either upper respiratory tract irritation or acute CNS depression in either of these studies, making 1200 mg/m^3 the no effect concentration for complex aliphatic solvents with low aromatic content. Ernstgard and colleagues compared the irritant and acute CNS effects of regular "white spirit" and a complex de-aromatized hydrocarbon solvent. In initial studies Ernstgard et al. (2009a) exposed volunteers in 10 minute intervals to vapor levels increasing from 0.5 to 600 mg/m^3. The authors reported that the median ratings for both irritation and acute CNS effects were "hardly at all" for the de-aromatized solvent, and none of the differences was statistically significant. As discussed in more detail below, the ratings were a bit higher for the regular "white spirit" with small but statistically significant differences recorded in the 500 and 600 mg/m^3 groups. In a subsequent study, volunteers were exposed for 4 hours to regular "white spirit" and the corresponding de-aromatized solvent at 100 and 300 mg/m^3. There was a statistically significant increase in eye irritation in the volunteers exposed to regular "white spirit" at 300 mg/m^3, but there were no effects in the study of the de-aromatized solvent. According to the subjective VAS ratings employed in the study, reports of eye discomfort ranged from "not at all" to slightly higher than "hardly at all". Furthermore, the authors concluded that their data supports the "low potency of white spirits to cause reports of eye irritation at moderate concentrations". It should be noted that the study included detailed pulmonary function tests and acoustic rhinometry parameters for both substances and at both exposure concentrations. The authors reported no exposure-related effects. In an additional study that included a more detailed assessment of nervous system effects, Juran et al. (2014) reported that neither white spirit nor the

equivalent de-aromatized solvent produced statistically significant effects at exposure levels up to 300 mg/m^3. In a study of n-decane, Kjaergaard et al. (1989) exposed 63 human subjects at levels up to 100 µl/L (which the authors reported was equivalent to 582 mg/m^3). Although the subjects were able to distinguish odor and knew when they were being exposed to test material, there was little indication of irritation or acute CNS effects. The only parameters that were considered to be dose-related were olfactory index, odor intensity, and mucous membrane irritation; however, the statistical association of mucous membrane irritation with exposure was weak and the results were considered inconclusive. In short, these studies provide evidence that the de-aromatized hydrocarbon solvents and their constituents are less irritating than the corresponding aromatic-containing substances. There is no evidence that the low aromatic and/or de-aromatized solvents produce ocular or upper respiratory tract irritation or acute CNS effects at levels up to approximately 1200 mg/m^3.

Aromatic Solvents

The occupational exposure recommendations for the alkylbenzenes and the solvents containing them were derived initially from occupational experience and volunteer testing. Battig et al. (1956) summarized observations of 27 painters who had used a commercial solvent containing 80% trimethylbenzene isomers at levels ranging from approximately 10 – 60 ppm (50-300 mg/m^3). Reported symptoms included headaches, feelings of tiredness during and after the shift, bronchitis with expectoration and coughing, bleeding of nose and gums with hematomas and reduced coagulation times, and a reduction in the number of erythrocytes. Animal studies confirmed the upper respiratory irritation and hematological effects (Battig et al., 1958), but hematological effects were not observed in subsequent studies with similar solvents (Nau et al., 1996; Clark et al., 1989), leading one author to suggest that the solvent used in the 1950s may have contained low levels of benzene as a contaminant (Gerarde, 1960). For comparative purposes, solvents of this type now contain < 10 ppm benzene (McKee et al. 2015). Based on their initial observations, Battig et al. (1958) recommended 35 ppm (175 mg/m^3) as an 8 hour time weighted average occupational exposure limit. Based on data from volunteer studies, Nau et al. (1966) recommended 50 ppm (250 mg/m^3). After consideration of these data, the ACGIH® adopted a value of 25 ppm (125 mg/m^3) as an 8 hour time-weighted average (TLV®). In tests of a similar solvent Carpenter et al. (1977a) reported that irritation of the throat was observed at 190 mg/m^3 but that no effects were observed at 100 mg/m^3, providing empirical support for the ACGIH TLV® of 25 ppm (125 mg/m^3). In 1994 the Scientific Committee for Occupational Exposure Limits (SCOEL) recommended an occupational exposure limit of 20 ppm (100 mg/m^3) based primarily on results of repeated inhalation studies in rats. This recommendation was then confirmed through human testing by three independent research organizations; all of whom reported that in periods ranging from 2 to 8 hours, and over an exposure range of 5 mg/m^3 to 150 mg/m^3, there were no signs of irritation, CNS depression or any other clinical evidence of effects from exposure at these levels (Jarnberg et al., 1996; 1997; Kostrzewski et al., 1997; Jones et al., 2006). In summary, independent expert groups using quite different methods, developed similar occupational exposure levels for alkylated benzenes, and three independent groups then used volunteer studies to demonstrate that exposure at the recommended levels was not associated with adverse effects.

In studies of two ring aromatics, Robbins (1951) reported that there were signs of ocular irritation in humans exposed to naphthalene at levels greater than 15 ppm (75 mg/m^3). Nau et al. (1966) reported that for C11-C12 aromatics, the minimum concentration which produced lacrimation and/or irritation of the eyes, skin, or mucus lining was 19 ppm (approximately 100 mg/m^3). Based in part on these data, the ACGIH® recommended an 8 hour TLV of 10 ppm (50 mg/m^3). As SCOEL recommended the same value, 50 mg/m^3 was used as a Substance Specific Value (SSV) in the reciprocal calculation formula to differentiate naphthalene from other aromatic solvent constituents. In more recent years, evidence that exposure to naphthalene by inhalation could produce pulmonary alveolar/bronchiolar adenomas in female mice and nasal tumors in rats (Abdo et al., 1992; 2001; Adkins et al., 1986; NTP 1992; 2000) has led to reconsideration of the occupational exposure recommendations. Complicating the process is that the rodent tumors may have been secondary to a cytotoxic process (e.g., North et al., 2008). As naphthalene is more irritating to the upper airways of rodents than humans, the potential for species-specificity must be considered (Buckpitt et al., 2002; Morris and Buckpitt, 2009). The ACGIH® had proposed a reduction in the TLV® for naphthalene, but reversed itself and continues to recommend 50 mg/m^3. SCOEL withdrew its Indicative Occupational Exposure Limit (IOELV) of 50 mg/m^3 but did not recommend an alternative value. In Germany the AGS reduced the occupational exposure limit for naphthalene to 0.5 mg/m^3, but has tabled that limit pending completion of an investigation of the irritating effects of naphthalene under occupational conditions. In short, there appears to be a consensus that protection from the irritant effects of naphthalene would also protect against other effects, but there is no agreement on the level of protection required.

The situation as it pertains to methylnaphthalenes is similar, but there is less data. Some fraction of methylnaphthalenes are, like naphthalene, metabolized by ring oxidation, so similar metabolites are expected. There is also evidence that methylnaphthalenes are upper respiratory irritants in rodents; in fact the ACGIH TLV® for methylnaphthalenes (apparently the only regulatory value for these substances) is 0.5 ppm (approximately 2.5 mg/m^3) based on sensory irritation tests of the two isomers in mice (Korsak et al., 1998). As human studies provide evidence that the methylnaphthalene isomers may be more irritating to rodents than to humans (Medeiros et al., 2000), it would seem reasonable to either reduce the guidance value for 2 ring aromatics to 50 mg/m^3, or to conduct a volunteer study with the methylnaphthalene isomers to provide additional information if a value separate from that of naphthalene seems warranted.

Complex Aliphatic/Aromatic Solvents

As previously noted, the first complex hydrocarbon solvent, Stoddard solvent (also known as white spirit or mineral spirit), a solvent containing approximately 80% aliphatic and 20% aromatic constituents, was introduced in the 1920s. Less volatile, low aromatic solvents became commercially available in Europe in the early 1980s, and, since their introduction, have replaced comparable solvents with higher aromatic levels in many commercial applications. In recent years, most of the human studies have involved either aromatic solvents or higher molecular weight, low aromatic solvents, in particular comparisons of the complex aliphatic/aromatic solvents to the comparable low aromatic grades as discussed above.

In one of the first volunteer studies of complex aliphatic/aromatic solvents, Nelson et al. (1943) reported studies to assess the irritating effects of several substances in studies in which groups of 10 volunteers were exposed for 3 to 5 minutes at increasing concentrations. They reported that "Stoddard's Solvent" produced no marked evidence for irritation at levels up to 400 ppm (approximately 2000 mg/m^3) although they considered that level too high for an 8 hour occupational exposure limit.

Astrand et al. (1975) reported that in preliminary information of humans exposed to "white spirit" at varying levels, there was evidence of severe discomfort and acute CNS effects at 5000 mg/m^3 with symptoms at a lesser degree at 2500 mg/m^3. The number of subjects and the length of the exposure periods were not specified. In a related study, Gamberale et al. (1975) exposed groups of 7 subjects to "white spirit" for 30 minutes at levels ranging from 625 to 2500 mg/m^3 and found no evidence that either subjective reactions or performance had been affected. In a second experiment the authors reported that effects on reaction time and probably impaired short term memory were associated with exposures of 4000 mg/m^3. Carpenter et al. (1975c) exposed volunteers in groups of 6 to "Stoddard solvent" at levels of 140, 850, or 2700 mg/m^3 and reported that 140 mg/m^3 was a no effect concentration, that there was slight evidence of throat irritation at 850 mg/m^3, and that at 2700 mg/m^3, there was evidence of ocular and upper respiratory tract irritation and acute CNS depression. Carpenter et al. (1975c) concluded that their data supported the ACGIH TLV® of 200 ppm (1150 mg/m^3) that had been published in 1971. However, by the time the Carpenter paper had been published, the ACGIH® had revised its TLV® downwards to 100 ppm (550 mg/m^3). Since that time, at least 8 additional studies have been conducted to assess whether there is any evidence of irritation and/or acute CNS effects at exposure levels approximating recommended occupational exposure levels. Some of these studies also assessed the potential for corresponding low aromatic solvents to produce effects. The majority of these studies reported that there were no, or at the most minimal, effects at recommended levels. More specifically, Cohr et al. (1980) compared responses of naïve (students) and previously-exposed (painters) subjects to "white spirit" vapor for 7 hours at concentrations ranging from 34 to 400 ppm (approximately 170 to 2000 mg/m^3). The number of subjects was not specified. The students reported upper respiratory tract irritation at levels $\geq$ 1000 mg/m^3 and evidence of acute CNS effects at levels $\geq$ 2000 mg/m^3. The painters reported upper respiratory tract irritation and acute CNS effects at levels of 500 mg/m^3. Most of these effects resolved quickly at the end of the exposure period. In neurobehavioral tests, the students were reported to have demonstrated statistically significant decrements in all of the performance tests at levels $\geq$ 500 mg/m^3 but apparently showed no effects in tests of memory function at levels up to 2000 mg/m^3. The painters on the other hand, had no decrements in performance tests at exposure levels up to 500 mg/m^3 (the highest level tested in this group), but did exhibit effects on memory at levels of either 250 or 500 mg/m^3. The authors were unable to determine whether the effects on memory in the painters were due to solvent exposure or age related effects. Hastings et al. (1982) exposed 25 subjects to "Stoddard solvent" for 30 minutes at 600 mg/m^3 and concluded that, at that level, there was no evidence for significant irritation or acute CNS depression. Pedersen and Cohr (1984) exposed 12 subjects to "white spirit" for 6 hours at approximately 600 mg/m^3 and found no evidence of either upper respiratory tract irritation or acute CNS effects. Jarnberg et al. (1997) exposed 7 volunteers to "white spirit" for 2 hours at 300 mg/m^3 and reported no indication of irritation or acute central nervous system depression. Lammers et al. (2007) exposed 12 volunteers for 4 hours to "white spirit" at

approximately 500 mg/m^3, and reported that there was no objective evidence of irritation but that there were small but statistically significant decrements in two measures of neurobehavioral performance. Ernstgard et al. (2009a) exposed 8 volunteers to white spirit at increasing levels between 0.5 and 600 mg/m^3. They reported that reports of irritation and evidence of CNS effects increased with increasing exposure levels, with the differences becoming statistically significant at 500 mg/m^3. Ernstgard et al. (2009b) exposed 12 volunteers for 4 hours to "white spirit" at levels of 300 mg/m^3 and reported that there was a significant elevation in eye irritation although the overall degree was in the range between "hardly at all" and "somewhat". Juran et al. (2014) exposed 12 volunteers to "white spirit" for 4 hours at levels of 100 and 300 mg/m^3 and reported that there was no evidence of CNS effects at these levels. Taking these data as a whole, it appears that for the aliphatic/aromatic hydrocarbon solvents upper respiratory tract irritation is a more sensitive indicator of effects than acute CNS effects, and that, overall, 300 mg/m^3 is a no effect level in humans for all effects, with subtle effects being reported over the range of approximately 400-600 mg/m^3. It should be noted that the aromatic constituents are the primary contributors to the irritant and acute CNS effects, and, as the levels of aromatics in commercial solvents can vary over a range up to a maximum of 25%, small differences in no effect concentrations could be due to the compositional variability.

COMPARISONS OF PREDICTED NO EFFECT LEVELS TO HUMAN OBSERVATIONS

In 2005, McKee et al. proposed a method to calculate occupational exposure limits for complex hydrocarbon solvents based on the reciprocal calculation formula from the ACGIH® (Appendix H). As a basis for the calculation, constituents were separated into four groups and assigned guidance values; specifically C5-C8 aliphatics, 1500 mg/m^3; C9-C15 aliphatics, 1200 mg/m^3; C7-C8 aromatics, 200 mg/m^3; and C9-C15 aromatics, 100 mg/m^3 (n-hexane and naphthalene were assigned separate values but are not directly relevant to these examples). With respect to acute central nervous system effects, as discussed above, among the C5-C8 aliphatic constituents, the lowest no effect levels were approximately 14000 mg/m^3 for alkanes, and 4200 for cycloparaffins. Among the C9-C15 aliphatics, the lowest no effect levels were 1200 mg/m^3 for alkanes and > 5000 mg/m^3 for cycloparaffins. For the C9-C13 aromatics, the lowest no effect level was 200 mg/m^3. The PBPK models predicted that at equivalent external exposure levels, alkane levels in the central nervous system would be similar to humans and rats, whereas levels of cycloparaffins in humans would be approximately twice the levels in rats. Assuming the degree of acute CNS effects in humans and rats to be similar at equivalent concentrations of hydrocarbons in the central nervous system, and using the no effect levels from the studies in rats with the correction factors from the PBPK models, theoretical human no effect levels can be calculated for solvents of differing composition. These predictions can then be compared to the results of volunteer studies of hydrocarbon solvents of known composition. Although many of the solvents tested by Carpenter and associates are no longer representative of products in commerce, the human evidence is well suited to this purpose. Accordingly, 3 examples were chosen, rubber solvent, Varnish Makers' and Painters (VM&P) naphtha, and Stoddard solvent.

Rubber Solvent – As described by Carpenter et al. (1975d) "rubber solvent" was an aliphatic solvent containing 42% C6-C7 alkanes, 54% C6-C7 cycloparaffins, and 3% C7 aromatics (i.e., toluene). Using a reciprocal procedure and assuming additivity of effects (and using the ACGIH TLV® of 75 mg/m^3 for toluene) the predicted no effect level in humans is:

(0.42/14000) + (0.54/2100) + (0.03/75) = 1/predicted no effect level = 1449 mg/m^3.

In tests of volunteers exposed to "rubber solvent" at levels of 1700, 3100, 6700 and 8100, the concentration considered most appropriate as an occupational standard was 1700 mg/m^3.

A theoretical occupational exposure level for this solvent calculated in accordance with the reciprocal calculation procedure is:

(0.97/1500) + (0.03/75) = 1/occupational exposure limit = 952 mg/m^3.

VM&P naphtha – Carpenter et al. (1975b) described this solvent as containing 26% C6-C8 alkanes, 29% C9-C11 alkanes, 21% C5-C8 cycloparaffins, 9% C9-C11 cycloparaffins, and 12% C7-C10 aromatics. Using the same assumptions (but with a common GGV of 100 mg/m^3 for all aromatics) given above, the predicted human no effect concentration is:

0.26/14000 + 0.29/1200 + 0.21/2100 + 0.09/2500 + 0.12/100 = 1/predicted human no effect level = 627 mg/m^3.

The no effect level derived from human testing is 1400 mg/m^3.

The occupational exposure limit, calculated using the reciprocal formula is:

0.26/1500 + 0.29/1200 + 0.21/1500 + 0.09/1200 + 0.12/100 = 1/occupational exposure limit = 571 mg/m^3.

Using the Guidance Values from TRGS 900, the calculated occupational exposure level is:

0.26/1500 + 0.29/600 + 0.21/1500 + 0.09/600 + 0.12/100 = 476 mg/m^3.

Stoddard Solvent – According to Carpenter et al. (1975c) the test sample of Stoddard solvent contained 48% C9-C12 alkanes, 7% C7-C8 cycloaraffins, 31% C9-C12 cycloparaffins, and 14% C9 aromatics. The predicted no effect level in humans is:

0.48/1200 + 0.07/2100 + 0.31/2500 + 0.14/100 = 1/human no effect level = 512 mg/m^3.

As summarized above, the lowest reported no effect level for acute effects was 270 mg/m^3 with 850 mg/m^3 as a low effect level with the evidence from all studies suggesting a most likely no effect level in the range of 400 – 500 mg/m^3.

The calculated occupational exposure limit is:

0.79/1200 + 0.07/1500 + 0.14/100 = 1/calculated occupational exposure limit = 476 m g/m^3.

Using the guidance values from TRGS 900, the calculation is:

0.79/600 + 0.07/1500 + 0.14/100 = 364 mg/m^3.

As shown in these three examples, the human no effect level was reasonably predicted from information derived from animal data and adjusted using PBPK modeling. The predicted and empirically derived no effect levels in humans were similar, and higher than the occupational exposure levels calculated using the reciprocal procedure following the

method proposed by McKee et al (2005) and described in Appendix H of the ACGIH TLV® manual.

SUMMARY AND CONCLUSIONS

Toxicological studies of hydrocarbon solvents have shown that in most cases repeated exposures produce minimal systemic effects (e.g., McKee et al., 2015). The findings most commonly reported are liver enlargement without pathological changes or elevated levels of marker enzymes. When the animals are held without treatment during a post-exposure period, the liver weights return to values consistent with the untreated control animals. These results provide evidence that the liver weight increases reflect a compensatory process related to increased metabolic demands, and are, therefore, adaptive rather than adverse (Maranpot et al., 2010). In studies in which rats are exposed to isoparaffinic and/or cycloparaffinic hydrocarbons, α2u-globulin-mediated nephropathy is often observed. This process is male rat specific and not relevant to humans (US EPA, 1991; Swenberg and Lehman-McKeeman, 1998). Finally, in some studies, there are small but statistically significant reductions in hemoglobin content/and or hematocrit levels. The underlying mechanism is unknown, but as the differences are within normal physiological ranges, the changes are not considered to be toxicologically important (Car et al., 2006). With this as background, the effects that are most useful in setting occupational exposure levels for hydrocarbon solvents are upper respiratory tract irritation and acute CNS depression, and, indeed, these effects are commonly cited as the basis for ACGIH TLV® recommendations. There are some constituents, n-hexane and diethyl- and triethylbenzene isomers with ethyl groups on adjacent carbons, that can produce chronic neurotoxic effects, but these effects require the production of constituent-specific metabolites. Other hydrocarbon solvent constituents, including those with very similar structures, do not produce the neurotoxic metabolites at toxicologically relevant levels.

As discussed above, upper respiratory tract irritation is often assessed in human volunteer studies. Irritation can be assessed fairly rapidly since, as discussed by Ernstgard et al. (2009a), chemosensory irritation will often appear within 10 minutes. Acute CNS depression may require a longer exposure period to be fully expressed as the effects are related to hydrocarbon concentrations in the central nervous system which, although increasing rapidly from the onset of exposure, may not reach steady state for several hours. In the early days volunteers were often exposed for short periods of time over a range of concentrations to identify no effect levels, a strategy which was probably an efficient means to assess irritation but might have underestimated the potential for acute CNS effects as the exposure periods were not long enough to achieve steady state levels of hydrocarbons in the central nervous system. In recent years, volunteers have been exposed for longer periods of time, but usually not at levels exceeding occupational exposure recommendations. Thus, the older data is more suited to setting occupational exposure limits whereas more recent studies have been used to confirm recommendations derived in other ways, usually from animal data.

Because of the ethical limitations of human testing, animal studies are conducted to the extent possible. The animals can be exposed to relatively high levels under controlled conditions, but there is always the issue as to how to relate the results to humans exposed at lower levels. As summarized, the acute CNS properties of a range of hydrocarbon

constituents was assessed in rats, and PBPK models were then used to compare the human and animal data, assuming the degree of acute CNS effects is similar in the two species at equivalent brain concentrations (Hissink et al., 2007; 2009). These data were then used to predict human no effect levels which could then be compared to the results of volunteer studies of solvents of known composition as well as to the occupational exposure limits for the same solvents calculated in accordance with the reciprocal calculation procedure proposed by McKee et al. (2005). As demonstrated, the calculations predicted human no effect levels similar or below those obtained experimentally, and the calculated occupational exposure levels were even lower.

In summary, this analysis shows that the occupational exposure limits calculated following the recommendations of McKee et al. (2005) protect against the most sensitive of the human effects. In this respect the proposal which had previously been based on consensus occupational exposure limits is shown to be consistent with the most current scientific information, and to also have been validated by comparison to human experience.

REFERENCES

Abdo, K., Eustis, S., McDonald, M., Jokien, M., Adkins, B., Haseman, J., 1992. Naphthalene: a respiratory toxicant and carcinogen for mice. Inhalation Toxicology 4, 393-409.

Abdo, K., Grumbein, S., Chou, B., Herbert, R., 2001. Toxicity and carcinogenicity in F344 rats following 2 years of whole body exposure to naphthalene vapors. Inhalation Toxicology 13,931-950.

ACGIH, 2015. Appendix H. Reciprocal Calculation Method for Certain Refined Hydrocarbon Solvent Mixtures. TLVs and BEIs. Threshold Limit Values (TLVs) and Biological Exposure Indices (BEIs). ACGIH, Cincinnati, OH.

Adenuga, D., Carrillo, J-C., McKee, R., 2014a. The sub-chronic oral toxicity of 1,3,5-trimethylbenzene in Sprague-Dawley rats. Regulatory Toxicology and Pharmacology 69, 143-153.

Adenuga, D., Carrillo, J-C., McKee, R., 2014b. The sub-chronic toxicity of dearomatized hydrocarbon solvents in Sprague-Dawley rats. Regulatory Toxicology and Pharmacology 70, 659-672.

Adkins, B., Van Stee, E., Simons, J., Eustis, S., 1986. Oncogenic response of strain A/J mice to inhaled chemicals. Journal of Toxicology and Environmental Health 17, 311-322.

Astrand, I., Kilbom, A., Ovrum, P., 1975. Exposure to white spirit 1. Concentration in alveolar air and blood during rest and exercise. Scandinavian Journal of Work, Environment and Health 1, 15-30.

Battig, K., Grandjean, E., Turrian, V., 1956. Health damage after continuous exposure to trimethylbenzene in a painting shop. Z. Prev Med. 1, 389-403

Battig, K., Grandjean, E., Rossi, I., Rickenbacher, J., 1958. Toxicological investigations concerning trimethylbenzene. Arch Gewerbepath Gewerbehyg. 16, 555-566.

Blake, M., 1971. Temperment and time of day. In: Colquhoun, W. (Ed.), editor. Biological rhythms and human performance. Academic Press, London, pp 109-148.

Boyes, W., Oshiro, W., El-Masri, H., Degn, L., Bercegeay, M., Krantz, Q., Bushnell, P., 2010. Acute inhalation of 2,2,4-trimethylpentane alters visual evoked potential and signal detection behavior in rats. Neurotoxicology and Teratology 32, 525-535.

Bowen, S., Balster, R., 1998. The effects of inhaled isoparaffins on locomotor activity and operant performance in mice. Pharmacol Biochem Behav. 61, 271-280.

Buckpitt, A, Boland, B., Isbell, M., Morin, D., Shultz, M., Baldwin, R., Chan, K., Karlsson, L., Lin, C., Taff, A., West, J., Fanucchi, M., van Winkle, L., Plopper, C. Naphthalene-induced respiratory tract toxicity. Metabolic mechanisms of toxicity. Drug Metabolism Reviews. 34, 791-820.

Car, B., Eng, V., Everds, N., Bounous, D., 2006. Clinical pathology of the rat. In: Sukow, M., Weisbroth, S., Franklin, C. (Eds.), The Laboratory Rat. 2nd edition, Elsevier Inc., Burlington MA, pp. 127-146.

Carpenter, C., Kinkead, E., Geary, D., Sullivan, L., King, J., 1975a. Methodology. Toxicology and Applied Pharmacology 32, 246-262.

Carpenter, C., Kinkead, E., Geary, D., Sullivan, L., King, J., 1975b. Petroleum hydrocarbon toxicity studies II. Animal and human response to vapors of Varnish Makers' and Painters Naphtha. Toxicology and Applied Pharmacology 32, 263-281.

Carpenter, C., Kinkead, E., Geary, D., Sullivan, L., King, J., 1975c. Petroleum hydrocarbon toxicity studies III. Animal and human response to vapors of Stoddard solvent. Toxicology and Applied Pharmacology 32, 282-297.

Carpenter, C., Kinkead, E., Geary, D., Sullivan, L., King, J., 1975d. Petroleum hydrocarbon toxicity studies IV. Animal and human response to vapors of rubber solvent. Toxicology and Applied Pharmacology 33, 526-542.

Carpenter, C., Kinkead, E., Geary, D., Sullivan, L., King, J., 1975e. Petroleum hydrocarbon toxicity studies V. Animal and human response to vapors of mixed xylenes. Toxicology and Applied Pharmacology 33, 543-558.

Carpenter, C., Kinkead, E., Geary, D., Sullivan, L., King, J., 1975f. Petroleum hydrocarbon toxicity studies VI. Animal and human response to vapors of "60 solvent". Toxicology and Applied Pharmacology 34, 374-394.

Carpenter, C., Kinkead, E., Geary, D., Sullivan, L., King, J., 1975g. Petroleum hydrocarbon toxicity studies VII. Animal and human response to vapors of "70 solvent". Toxicology and Applied Pharmacology 34, 395-412.

Carpenter, C., Kinkead, E., Geary, D., Sullivan, L., King, J., 1975h. Petroleum hydrocarbon toxicity studies VIII. Animal and human response to vapors of "140 flash aliphatic solvent". Toxicology and Applied Pharmacology 34, 413-429.

Carpenter, C., Kinkead, E., Geary, D., Sullivan, L., King, J., 1976a. Petroleum hydrocarbon toxicity studies IX. Animal and human response vapors of "80 thinner". Toxicology and Applied Pharmacology 36, 409-425.

Carpenter, C., Kinkead, E., Geary, D., Sullivan, L., King, J., 1976b. Petroleum hydrocarbon toxicity studies X. Animal and human response to vapors of "50 thinner". Toxicology and Applied Pharmacology 36, 427-442.

Carpenter, C., Kinkead, E., Geary, D., Sullivan, L., King, J., 1976c. Petroleum toxicity studies XI. Animal and human response to vapors of deodorized kerosene. Toxicology and Applied Pharmacology 36, 443-446.

Carpenter, C., Kinkead, E., Geary, D., Sullivan, L., King, J., 1976d. Petroleum toxicity studies XII. Animal and human response to vapors of "40 thinner". Toxicology and Applied Pharmacology. 36, 457-472.

Carpenter, C., Kinkead, E., Geary, D., Sullivan, L., King, J., 1976e. Petroleum toxicity studies XIII. Animal and human response to vapors of toluene concentrate. Toxicology and Applied Pharmacology. 36, 473-490.

Carpenter, C., Kinkead, E., Geary, D., Sullivan, L., King, J., 1977a. Petroleum toxicity studies XIV. Animal and human response to vapors of high aromatic solvent. Toxicology and Applied Pharmacology 41, 251-260.

Carpenter, C., Kinkead, E., Geary, D., Sullivan, L., King, J., 1977b. Petroleum toxicity studies XV. Animal and human response to vapors of "high naphthenic solvent". Toxicology and Applied Pharmacology 41, 251-260.

Carpenter, C., Kinkead, E., Geary, D., Sullivan, L., King, J., 1977c. Petroleum toxicity studies XVI. Animal and human response to vapors of naphthenic aromatic solvent. Toxicology and Applied Pharmacology 41, 261-270.

Carpenter, C., Kinkead, E., Geary, D., Sullivan, L., King, J., 1978. Petroleum hydrocarbon toxicity studies XV. Animal and human response to vapors of n-nonane. Toxicology and Applied Pharmacology 44, 53-62.

Carrillo, J-C., Adenuga, M., McKee, R., Roth, R., Steup, D., Simpson, B., 2013. The sub-chronic toxicity in rats of isoparaffinic solvents. Regulatory Toxicology and Pharmacology 67, 446-455.

Carrillo, J-C., Adenuga, M., McKee, R., 2014. The sub-chronic toxicity of regular white spirit in rats. Regulatory Toxicology and Pharmacology 70, 222-230.

Christoph, G., Kelly, D., Krivanek, N., 2000. Acute inhalation exposure to cyclohexane and schedule-controlled operant performance I rats: comparison to D-amphetamine and chlorpromazine. Drug and Chemical Toxicology 23, 539-553.

Clark, D., Butterworth, S., Martin, J., Roderick, H., Bird, M., 1989. Inhalation toxicity of high flash aromatic naphtha. Toxicol. Ind. Health 5, 415-428.

Cohr, K., Stockholm, J., Bruhn, P., 1980. Neurologic response to white spirit exposure. In: Holmstedt, B., Lauwerys, A., Mercier, M., Roberfroid, M. (Eds). Mechanisms of Toxicity and Hazard Evaluation. Elsevier/North Holland Biomedical Press, Amsterdam, the Netherlands, pp. 95-102.

Cushman, J., Norris, J., Dodd, D., Darmer, K., Morris, C., 1995. Chronic inhalation toxicity and neurotoxicity assessment of cumene in Fischer 344 rats. Journal of the American College of Toxicology 14, 129-147.

Douglas, J., McKee, R., Cagen, S., Schmitt, S., Beatty, P., Swanson, M., Schreiner, C., Ulrich, C., Cockerell, B., 1993. A neurotoxicity assessment of high flash aromatic naphtha. Toxicology and Industrial Health 9, 1047-1057.

Egan, G., Spencer, P., Schaumberg, H., Murray, K., Bischoff, M., Scala, R. 1980. N-hexane "free" hexane mixture fails to produce nervous system damage. Neurotoxicology 1, 515-524.

Ernstgard, L., Lind, B., Johanson, G. 2009a. Acute effects of exposure to vapours of standard and dearomatized white spirits in humans. Dose-finding study. Journal of Applied Toxicology 29, 263-274.

Ernstgard, L., Iregren, A., Juran, S., Sjogren, B., van Thriel, C., Johanson, G. 2009b. Acute effects of exposure to vapours of standard and dearomatized white spirit in humans. 2 Irritation and inflammation. Journal of Applied Toxicology 29, 263-274.

Frontali, N., Amantini, M., Spagnolo, A., Guarcini, A., Saltari, M., Brugnone, F., Perbelini, L., 1981. Experimental neurotoxicity and urinary metabolites of the C5-C7 aliphatic hydrocarbons used as glue solvents in shoe manufacture. Clinical Toxicology 18, 1357-1367.

Gagnaire, F., Becker, M., De Ceaurriz, J., 1992. Alteration of brainstem auditory evoked potential in diethylbenzene and diacetylbenzene-treated rats. Journal of Applied Toxicology 12, 343-350.

Gagnaire, F., Marignac, B., De Ceaurriz, J., 1991. Possible involvement of 1,2-diacetylbenzene in diethylbenzene-induced neuropathy in rats. Journal of Applied Toxicology 11, 262-268.

Gagnaire, F., Marignac, B., De Ceaurriz, J., 1990. Diethylbenzene-induced sensorimotor neuropathy in rats. Journal of Applied Toxicology 10, 105-112.

Gagnaire, F., Marignac, B., De Ceauriz, J., 1993. Triethylbenzene-induced sensorimotor neuropathy in rats. Journal of Applied Toxicology 13, 123-128.

Gamberale, F., Annwall, G., Hultengren, M., 1975. Exposure to white spirit II. Psychological functions. Scandinavian Journal of Work, Environment and Health 1, 31-39.

Gerarde, H. 1960. Toxicology and biochemistry of aromatic hydrocarbons. In: Elsevier Monographs on Toxic Agents, Browning, E. (Ed.), New York: Elsevier Publishing Company, pp 191-192.

Hastings, L., Cooper, G., Burg, W., 1982. Human sensory response to selected petroleum hydrocarbons In: The Toxicology of Petroleum Hydrocarbons, MacFarland, H., Holdsworth, H., MacGregor, J., Call, R., Kane, M. (Eds.), The American Petroleum Institute, Washington D.C., pp. 359-376.

Hau, K., Connell, D., Richardson, B., 2001. A study of the biological partitioning behavior of n-alkanes and n-alkanols in causing anesthetic effects. Regulatory Toxicology and Pharmacology 35, 273-279.

Hissink, A., Kruse, J., Kulig, B., Verwei, M., Muijser, H., Salmon, F., Leenhers, L., Owen, D., Lammers, J., Freidig, A., McKee, R., 2007. Model studies for evaluating the neurobehavioral effects of complex hydrocarbon solvents III. PBPK modeling of white spirit constituents as a tool for integrating animal and human test data. Neurotoxicology 28, 751-760.

Hissink, A., Kulig, B., Kruse, J., Freidig, A., Verwei, M., Muijser, H., Lammers, J., McKee, R., Owen, D., Sweeney, L., Salmon, F., 2009. Physiologically based pharmacokinetic modeling of cyclohexane as a tool for integrating animal and human test data. International Journal of Toxicology 28, 498-509.

IPCS, 1991. N-hexane. Environmental Health Criteria 122, World Health Organization, Geneva, Switzerland.

Jarnberg, J., Johanson, G., Lof, A., 1996. Toxicokinetics of inhaled trimethylbenzenes in man. Toxicology and Applied Pharmacology 140, 81-288.

Jarnberg, J., Johanson, G., Lof, A., Stahlbom, B., 1997. Inhalation toxicokinetics of 1,2,4-trimethylbenzene in volunteers: comparison between exposure to white spirit and 1,2,4-trimethylbenzene alone. Science of the Total Environment 199, 65-71.

Jones, K., Meldrum, G., Baird, E., Cottrell, S., Kaur, P., Plant, N., Dyne, D., Cocker, J., 2006. Biological monitoring for trimethylbenzene exposure: A human volunteer study and a practical example in the workplace. Annals of Occupational Hygiene 50, 591-598.

Juran, S., Johanson, G., Ernstgard, I., Iregren, A., van Thriel, G., 2014. Neurobehavioral performance in volunteers after inhalation of white spirits with higher and lower aromatic content. Archives of Toxicology 88, 1127-140.

Kim, M., Hashemi, S., Spencer, P., Sabri, M., 2002. Amino acid and protein targets of 1,2-diacetylbenzende a potent aromatic γ-diketone that induces proximal neurofiliamentous axonopathy. Toxicology and Applied Pharmacology 183, 55-65.

Kim, M., Sabri, M., Miller, V., Kayton, R., Dixon, D., Spencer, P., 2001. 1,2-Diacetylbenzene, the neurotoxic metabolite of a chromogenic aromatic solvent induces proximal neuropathy. Toxicology and Applied Pharmacology 177, 121-131.

Kjaergaard, S., Molhave, L., Pedersen, O., 1989. Human reactions to indoor air pollutants: n-decane. Environment International 15, 473-482.

Korsak, K., Majcherek, W., Rydzynski, K., 1998. Toxic effects of acute inhalation exposure to 1-methylnaphthalene and 2-methylnaphthalene in experimental animals. International Journal of Occupational Medicine and Environmental Health 14, 355-342.

Korsak, K., Rydzynski, K., 1996. Neurotoxic effects of acute and subchronic inhalation exposure to trimethylbenzene isomers (pseudocumene, mesitylene, hemimellitene) in rats. International Journal of Occupational Medicine and Environmental Health 9, 341-349.

Kostrzewski, P., Wiaderna-Brycht, A., Czerski, B., 1997. Biological monitoring of experimental human exposure. Science of the Total Environment 199, 73-81.

Lammers, J., Emmen, H., Muijser, H., Hoogendijk, E., McKee, R., Owen, D., Kulig, B., 2006. Model studies for evaluating the neurobehavioral effects of complex hydrocarbon solvents II. Neurobehavioral effects of white spirit in rat and human. Neurotoxicology 28, 736-750.

Lammers, J., Emmen, H., Muijser, H., Hoogendijk, E., McKee, R., Owen, D., Kulig, B., 2009. Neurobehavioral effects of cyclohexane in rat and human. International Journal of Toxicology 28, 488-497.

Lammers, J., Muijser, H., Owen, D., Kulig, B., McKee, R., 2011. Neurobehavioral effects of acute exposure to normal (n-) paraffins. International Journal of Toxicology 30, 47-58.

Lewis R., 1993. Hawley's Condensed Chemical Dictionary, 12th edition. John Wiley and Sons, New York.

McKee, R., Adenuga, M., Carrillo, J-C., 2015. Characterization of the toxicological hazards of hydrocarbon solvents. Critical Reviews in Toxicology 45: 2373-365.

McKee, R., Frank, E., Heath, H., Przygoda, R., Trimmer, G., Whitman, F., 1998. Toxicology of n-pentane (CAS no. 109-66-0). Journal of Applied Toxicology 18, 431-442.

McKee, R., Lammers, J., Hoogenijk, E., Emmen, H., Muijser, H., Barsotti, D., Owen, D., Kulig, B., 2006. Model studies for evaluating the acute neurobehavioral effects of complex hydrocarbon solvents 1. Validation of methods with ethanol. Neurotoxicology 27, 1064-1079.

McKee, R., Lammers, J., Muijser, H., Owen, D., 2011. Neurobehavioral effects of acute exposure to isoparaffinic and cycloparaffinic hydrocarbons. International Journal of Toxicology 30, 715-734.

McKee, R., Lammers, J., Muijser, H., Owen, D., Kulig, B., 2010. Neurobehavioral effects of acute exposure to aromatic hydrocarbons. International Journal of Toxicology 29, 277-290.

McKee, R., Medeiros, A., Daughtrey, W., 2005. A proposed methodology for setting occupational exposure limits for hydrocarbon solvents. Journal of Occupational and Environmental Hygiene 2, 524-542.

Medeiros, A., Daughtrey, W., Jarnot, B., Schmidt, R., Cain, W., 2000. Human sensory irritation and odor testing on a complex aromatic solvent. The Toxicologist 54, 190 (abstract).

Morris, J., Buckpitt, A., 2009. Upper respiratory tract uptake of naphthalene. Toxicological Sciences 111, 383-391.

National Toxicology Program. 1992. Toxicology and carcinogenicity studies of naphthalene (CAS number 91-20-3) in B6C3F1 mice (inhalation studies). NTP-TR-410; NIH Publication no. 92-31. US Public Health Services. National Toxicology Program, Rockville, MD.

National Toxicology Program. 2000. Toxicology and carcinogenicity studies of naphthalene (CAS no. 91-0-3) in F344/N rats (inhalation studies). National Institutes of Health Technical Report Series no. 500. US Department of Health and Human Services. National Toxicology Program. Rockville, MD.

Nau, C., Neal, J., Thornton, M., 1966. C9-C12 fractions derived from petroleum distillates. Archives of Environmental Health 12, 382-393.

Nilsen, O., Haugen, O., Zahlsen, K., Halgunset, J., Aarset, H., Eide, I., 1988. Toxicity of n-C9 to n-C13 alkanes in the rat on short term inhalation. Pharmacol Toxicol 62, 259-266.

North, W., Abdo, K., Benson, J., Dahl, A., Morris, J., Renne, R., Witschi, H., 2008. A review of whole-animal bioassays of the carcinogenic potential of naphthalene. Regulatory Toxicology and Pharmacology 51, S6-S14.

Ono, Y., Takeuchi, Y., Hisanaga, N., 1981. A comparative study on the toxicity of n-hexane and its isomers on the peripheral nerve. International Archives of Occupational and Environmental Health 48, 289-294.

Patty, F., Yant, W., 1929. Report of Investigations. Odor intensity and symptoms produced by commercial propane, butane, pentane, hexane and heptane. Department of Commerce, United States Department of Mines.

Pedersen, E., Cohr, K., 1984. Biochemical pattern in experimental exposure of humans to white spirit. 1. The effects of a 6 hours single dose. Acta Pharmacol et Toxicol 55, 317-324.

Robbins, M., 1951. Determination of naphthalene in air. AMA Archives of Industrial Hygiene and Occupational Medicine 4, 85-87.

Sabri, M., Hashemi, S., Laarev, M., Spencer, P., 2007. Axonopathy-inducing 1,2-diacetylbenzene forms adducts with motor and cytoskeletal proteins required for axonal transport. Neurochemical Research 32, 2152-2159.

Scientific Committee on Occupational Exposure Limits. 1994. SEG/SUM 34. Recommendation from Scientific Expert Group on Occupational Exposure Limits for Trimethylbenzenes.

Swenberg, J., Lehman-McKeeman, L., 1998. Alpha 2u-globulin associated nephropathy as a mechanism of renal carcinogenesis in male rats. In: Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis. IARC Scientific Publication 147. International Agency for Research on Cancer. Lyon, France.

Takeuchi, Y., Ono, Y., Hisanaga, N., Kotoh, J., Sugiura, Y., 1980. A comparative study on the neurotoxicity of n-pentane, n-hexane and n-heptane in the rat. British Journal of Industrial Medicine 37, 241-247.

U.S. Environmental Protection Agency, 1991. Alpha 2u-globulin: Association with chemically induced renal toxicity and neoplasia in the male rat. Risk Assessment Forum. EPA/625/3-91/019F, September, 1991.

Virtue, R., 1948. Observations on cyclopentane as a narcotic agent. Anesthesiology 10, 318-324.

World Medical Association, 1989. Declaration of Helsinki: Recommendations Guiding Medical Doctors in Biomedical Research Involving Human Subjects. Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964 and amended by the 19th World Medical Assembly, Tokyo, Japan, October, 1975, 35th World Medical Assembly, Venice, Italy, October 1983, the 41st World Medical Assembly, Hong Kong, September, 1989.

Zahlsen, K., Eide, I., Nilsen, A., Nilsen, O., 1992. Inhalation kinetics of C6 to C10 aliphatic, aromatic and naphthenic hydrocarbons in rat after repeated exposures. Pharmacology and Toxicology 71, 144-149.

Zahlsen, K., Eide, I., Nilsen, A., Nilsen, O., 1993. Inhalation kinetics of C8 to C10-1-alkenes and iso-alkanes in the rat after repeated exposures. Pharmacology and Toxicology 73, 163-168.

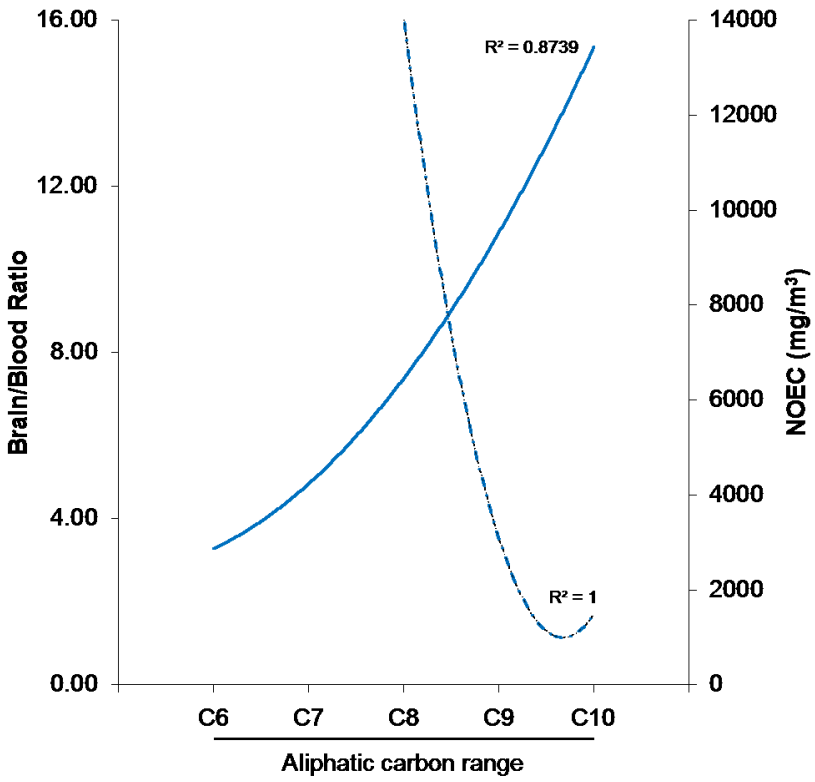
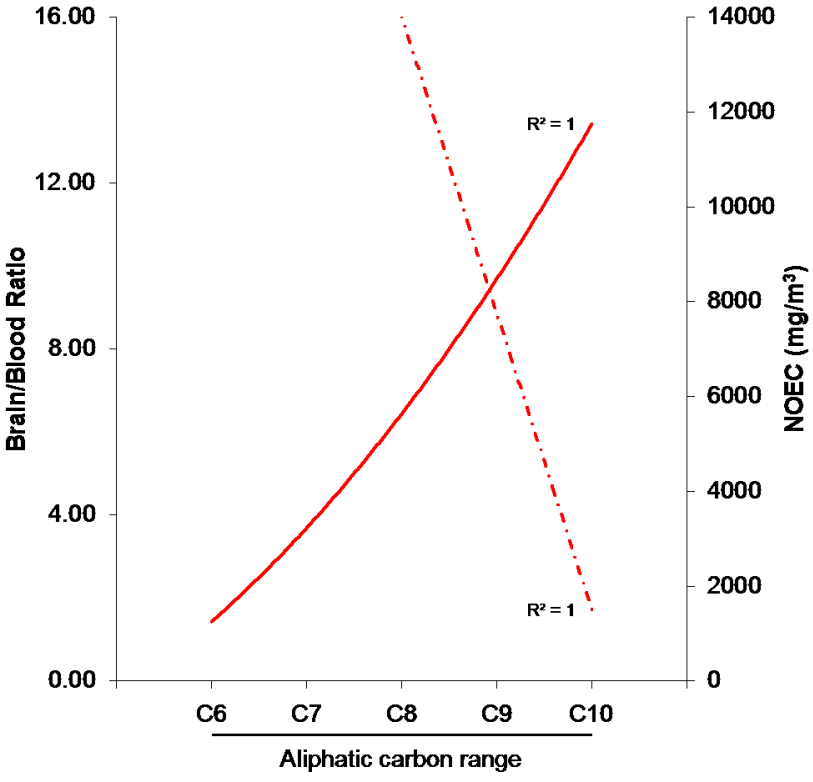
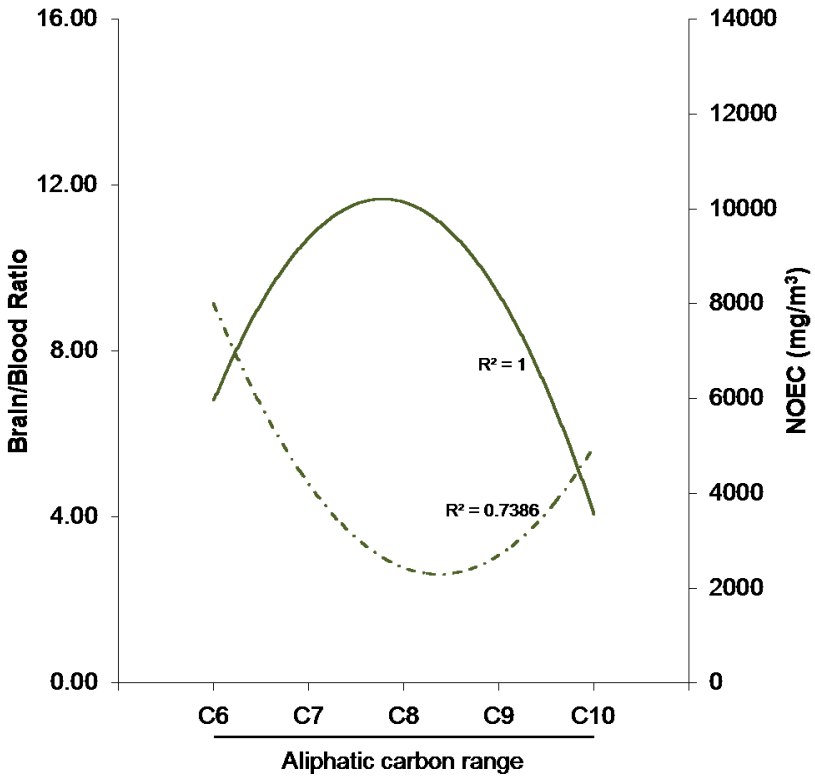


Figure 1: Correlation of brain/blood ratios and No-Effect-Levels for C6-C10 (a) n-paraffins (b) iso-paraffins and (c) cyclo-paraffins in rats. Solid lines – brain/blood ratios, dashed lines – NOECs. Brain and blood levels of hydrocarbons (µmol/kg) were obtained from rats exposed to 100 ppm of each n-paraffin on day 1 for 8 hrs (Zahlsen et al., 1992, 1993). NOECs were obtained from Lammers et al., 2009, 2011 and McKee et al., 2011. Note that each curve represents a polynomial regression fit to the actual data. R^2 correlation of coefficient values are indicated for each curve-fit.


16.00
12.00
8.00
4.00
0.00
Brain/Blood Ratio
14000
12000
10000
8000
6000
4000
2000
0
NOEC (mg/m³)
R² = 1
R² = 1
C6
C7
C8
C9
C10
Aliphatic carbon range


16.00
12.00
8.00
4.00
0.00
Brain/Blood Ratio
14000
12000
10000
8000
6000
4000
2000
0
NOEC (mg/m3)
R² = 1
R² = 0.7386
C6
C7
C8
C9
C10
Aliphatic carbon range