BEFORE THE
NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS

COMMENTS OF THE HYDROCARBON SOLVENTS PANEL OF THE AMERICAN CHEMISTRY COUNCIL IN RESPONSE TO NTP'S REQUEST FOR COMMENTS ON PROPOSED TESTING OF C9 ALKYLBENZENES

Notice of National Toxicology Program	)
Board of Scientific Counselors Meeting;	)
Request for Comments	)
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EXECUTIVE SUMMARY

The American Chemistry Council's Hydrocarbon Solvents Panel[1] represents the major US producers of hydrocarbon solvents including, C9 alkylbenzenes, of which trimethylbenzenes are a major component. The Panel appreciates the opportunity to comment on the NTP's research concept developed for evaluating the systemic effects of exposure to C9 alkylbenzenes, which will be a subject of the NTP Board of Scientific Counselors meeting, June 17-18, 2014.
We strive to ensure appropriate product stewardship, and, as part of our mission, address important science and public policy issues related to the hydrocarbon solvents industry, including this draft research concept.

As identified by the NTP, C9 alkylbenzenes (consisting primarily of isomers of trimethylbenzenes, ethyltoluenes and propylbenzenes) occur naturally in petroleum and are produced during petroleum refining. The major use for C9 alkylbenzenes is as a complex C9 aromatic hydrocarbon fraction that is used for gasoline-blending. The complex C9 aromatic fraction may also undergo additional refining steps to meet product specifications for use as solvents. The general population is primarily exposed to C9 alkylbenzenes as mixtures from automobile emissions.

As exposure to alkylbenzenes is primarily to mixtures of C9 isomers rather than to C9 constituents in isolation, we believe that for purposes of hazard characterization and risk assessment, the most useful information comes from studies of representative complex C9 aromatic hydrocarbon substances. This approach is supported by the similarities in the structure, physical/chemical properties and pathways of metabolism of the various C9 isomers. Further, data from toxicity studies of individual C9 isomers are similar to results of studies of complex C9 aromatic substances, providing further justification for the representative substance approach. We believe that the studies that have already been conducted provide sufficient data to characterize the carcinogenic, reproductive, developmental and neurotoxic potential of C9 alkylbenzenes such that additional studies are not warranted and will simply lead to a duplication of already existing data. We also note that the proposal to develop novel *in vitro* systems to assess the *in vitro* toxicity of C9 alkylbenzenes is not clear as the systemic effects of concern are best assessed using more relevant *in vivo* models.

[1] Members of the ACC Hydrocarbon Solvents Panel are Chevron Phillips, CITGO, ExxonMobil Chemical Company, and Sasol North America.

INTRODUCTION

The Hydrocarbon Solvents Panel (HSP) of the American Chemistry Council (ACC) appreciates the opportunity to comment on the proposal by the NTP to test C9 alkylbenzenes for carcinogenic potential and other toxicological effects.

In its proposal, the NTP asserts that there is insufficient information by which to characterize the toxicological hazards of individual C9 alkylbenzene isomers and that this additional information is needed to derive safe exposure levels. The HSP disagrees with this assessment for reasons discussed in more detail below. It is our view that the C9 alkylbenzenes collectively (i.e., trimethylbenzene, ethyltoluene and propylbenzene isomers) have already been well characterized both individually and collectively and that their toxicological properties are similar to each other and to those of more extensively tested complex C9 substances. Thus, we believe that further toxicology testing to collect hazard information on the individual C9 isomers is neither technically justified, nor a responsible use of laboratory animals. We further believe that a detailed assessment of the toxicological hazards of the individual isomers has no practical value in a regulatory or public health context. Because the C9 aromatics are manufactured and used as complex mixtures, human exposures are to the C9 isomers collectively. Thus, for regulatory and public health purposes, the approach that has been taken is to characterize the toxicological hazards of representative substances and to use those data as a basis for managing the potential hazards of C9 aromatics on a collective basis.

We would like to focus our comments on four specific points:

(i) Testing of representative complex C9 aromatic substances is a reasonable, pragmatic, and resource conservative approach to providing the data necessary for risk management.

(ii) The toxicological properties of representative complex C9 aromatics are similar to results of studies of individual isomers, confirming the validity of the complex substance approach.

(iii) Further hazard characterization tests are unnecessary as sufficient data are already available to address regulatory and public health needs, and

(iv) Further carcinogenesis tests specifically are unnecessary as the outcomes can be reasonably predicted from existing data, and further, that the anticipated results would be difficult to interpret in a public health context.

I. GENERAL COMMENTS

(1) Testing of representative complex C9 aromatic substances is a reasonable, pragmatic, and resource conservative approach to providing the data necessary for risk management.

The C9 alkylbenzenes are manufactured as a complex aromatic substance used for gasoline blending. Some of this material is also used for solvent applications. Because of the manufacturing process and commercial uses, humans are exposed to these substances as mixtures. Accordingly, the most efficient approach to assessing hazards is to comprehensively assess the toxicological properties of a representative mixture and to use the results to generically characterize the hazards of the individual constituents. Indeed, in 1985 the EPA adopted this approach and proposed testing on a commercial C9 aromatic material. Specifically the EPA stated that "the Agency agrees that testing the C9 fraction alone would most likely elucidate any potential problems that may result from exposures to the C9 fraction. Testing of the individual isomers does not appear necessary at this time in order to evaluate the risk posed by exposure to the C9 fraction and solvents containing it" (EPA, 1985).

Thus, the use of toxicological data from representative commercial products as a basis for assessing the hazards of the C9 aromatics collectively has been recognized as an effective means of developing the necessary data to manage the hazards of these substances for regulatory purposes.

(2) The toxicological properties of representative complex C9 aromatics are similar to results of studies of individual isomers, confirming the validity of the complex substance approach: There have been studies of representative complex C9 aromatic substances as well as studies of many of the isomers individually.[2]

Detailed summaries for the studies cited in this section are provided in the appendix. For comparison purposes, this section is divided into two parts; one summarizes studies on complex C9 aromatic substances and the second summarizes studies of constituents of complex C9 aromatic substances.

(2.1) Studies of representative complex C9 aromatic substances

[2] There are eight C9 aromatic molecules, three trimethyl benzene isomers (1,2,3 TMB; 1,2,4 TMB, 1,3,5 TMB), three ethyltoluene isomers (1,2 ET; 1,3 ET; 1,4 ET), and two possible propyl benzene isomers (n-propylbenzene, isopropyl benzene, commonly known as cumene). As these molecules have similar structures and similar physical/chemical properties, one would expect that they would also have similar toxicological properties.

In 1985, the EPA (EPA, 1985) determined that there were already existing data by which to assess the effects of repeated exposure by inhalation (published as Clark et al., 1989) and the potential to produce developmental toxicity in rats (Ungvary et al., 1983). The EPA considered that additional information was required for four other endpoints, specifically:

a) A battery of *in vitro* and *in vivo* mutagenicity tests (published as Schreiner et al., 1989). The test substance was not active in Salmonella; a forward mutation test conducted under *in vitro* conditions, chromosome aberration and sister chromatid exchange assays conducted under *in vitro* conditions, and an *in vivo* chromosome aberration test in rat bone marrow. The overall conclusion was that C9 aromatics were not genotoxic.

b) A developmental toxicity test in mice (published as McKee et al., 1990) in which there were no developmental effects at levels that were not maternally toxic (i.e., 100 ppm or approximately 500 mg/m^3). These data, along with previously published results provided evidence that C9 aromatics were not selective developmental toxicants but that there were effects, primarily developmental delays at maternally toxic levels.

c) A reproductive toxicity test in rats (published as McKee et al., 1990) in which there were no effects on fertility at the highest exposure level tested (1500 ppm or 7500 mg/m^3). These results provided evidence that C9 aromatics were not toxic to the reproductive system.

d) A subchronic neurotoxicity test (published as Douglas et al., 1993) in which repeated exposures for 90 days at levels up to 1500 ppm (7500 mg/m^3) had no persistent effects on motor activity or functional observations and did not produce any pathological changes in the nervous system.[3] These results provided evidence that C9 aromatics did not produce persistent neurological effects. Note that these studies did not assess acute neurological effects.

   The principal findings in these studies were that exposure of rats by inhalation at levels up to 7400 mg/m^3 for 90 days resulted in increased liver and kidney weights (without pathological changes) and small but statistically significant reductions in red blood cell counts. The no adverse effect concentration was 1800 mg/m^3. In a 12-month inhalation toxicity study, there were statistically significant increases in liver and kidney weights in male rats but no pathological changes in these organs. There were also some hematological changes; but, as these were not observed consistently throughout the study, they were judged to be incidental. In the absence of pathological effects, the authors considered that 1800 mg/m^3 was a no adverse effect concentration (NOAEC) in both the 90-day and 12-month studies.

[3] The emphasis is to distinguish persistent neurological effects from acute reversible central nervous system (CNS) effects. The specific objective of the study reported in Douglas et al. (1993) was to assess the potential of repeated exposure to C9 aromatics to cause persistent, irreversible neurological effects.

Following review of the above information, the EPA concluded that the objectives of the toxicology testing program had been satisfied and that no additional testing was necessary. In particular and directly relating to the objectives of the NTP proposal, the EPA concluded that further testing to assess carcinogenic potential was unnecessary (Merenda, 1988) as the data indicated that the complex C9 aromatic substance was not genotoxic and did not produce cumulative effects.

It should also be noted that in addition to the tests described above, a similar complex C9 aromatic substance did not affect fertility in a one-generation study in rats and did not produce developmental neurotoxicity (Lehotzky et al., 1985).

In summary: on the basis of the data available by 1990, it was evident that complex C9 solvents produced upper respiratory irritation, as well as acute central nervous system depression at high exposure levels that could result in the deaths of rats and mice. However, there was little evidence of systemic toxicity, no effects on fertility, no evidence of developmental effects other than developmental delays secondary to maternal effects, and no evidence of persistent neurological effects.

(2.2) Studies of individual C9 aromatic isomers

Since the completion and publication of the studies of commercial C9 aromatic substances, there have been a series of studies of individual TMB, ET, and propylbenzene isomers in which the potential for systemic toxicity and nervous system effects were assessed. Emphasis is placed on trimethylbenzenes, ethyltoluenes and propylbenzenes because they make up at least 80% of the commercial C9 aromatic substances. In particular, there were 3-month inhalation toxicity studies of 1,2,3 (Korsak et al., 2000a) and 1,2,4 (Korsak et al., 2000b) trimethylbenzene isomers, as well as developmental toxicity studies of 1,2,4 and 1,3,5 TMB isomers (Saillenfait et al., 2005), as well as a number of additional studies to further characterize acute and persistent nervous system effects (Korsak and Rydzynski, 1996; Gralewicz et al., 1997; Gralewicz and Wiaderna, 2001; Widaerna et al., 2002; McKee et al., 2010) and the potential for respiratory irritation (Swiercz et al., 2000). There are also a series of publications detailing studies of the potential for isopropylbenzene to cause subchronic (Cushman et al., 1995), neurotoxic (Cushman et al., 1995) or developmental effects (Darmer et al., 1997). In sum, these tests cover 5 of the 8 possible C9 aromatic structures.

Summarized results of the subchronic toxicity studies in comparison to those of a representative complex C9 aromatic substance are shown in Table 1. The commercial substance was tested by inhalation at levels of 1800, 3700 or 7400 mg/m^3 in a study in which rats were exposed 6 hours/day, 5 days/week for 13 weeks. The only effects were an increase in liver weight (without pathological changes or elevations in marker enzymes) and a small but statistically significant

reduction in red blood cell count. Of the 8 possible C9 isomers, there are subchronic inhalation toxicity studies of two TMB isomers, one ET isomer, and one propylbenzene isomer and a repeated oral toxicity study of the third TMB isomer. By comparison to the study of the complex C9 aromatic substance, the only differences found in the studies of individual isomers were (i) in a study of 4-ethyltoluene the authors reported a significant reduction in testicular weights (apparently without pathological changes) and (ii) in the cumene study there were kidney changes in male rats consistent with an α2u globulin-mediated process. The NOAECs obtained in these studies were in the range of 500 – 1800 mg/m^3 depending on the concentrations tested. In short, of 8 possible C9 isomers, 5 have been evaluated for subchronic toxicity with little evidence that these produce toxicologically important effects that are different from those of the complex C9 aromatic substance or that the individual isomer studies have substantially lower no adverse effect levels.

Results of developmental toxicity tests of C9 aromatic substances are shown in Table 2. The earliest developmental toxicity studies assessed the potential for effects in rats, rabbits and mice; but, because they involved continuous (i.e., approximately 24 hours/day) exposures, they are not directly comparable to those conducted later, although the data indicated that the C9 aromatic substances were not selective developmental toxicants in any of the species tested. A developmental toxicity study of a representative C9 aromatic substance in the mouse that was conducted under a protocol more similar to current study designs is included in Table 2 for comparative purposes. In that study, the mice were exposed at levels of 500, 2500 or 7500 mg/m^3. The highest exposure level resulted in significant maternal mortality and profound fetal effects. The 2500 mg/m^3 level produced some maternal toxicity as well as developmental delays but did not cause fetal death or malformation. The lowest exposure level (500 mg/m^3) was a no effect concentration (NOEC) for both maternal and fetal effects. Similar results were obtained in developmental toxicity studies in rats of two TMB isomers, one ET isomer and one propylbenzene isomer. In short, there are developmental toxicity data for 4 of 8 C9 isomers, and the results of these studies do not differ substantially from those obtained in studies of the representative complex substances.

Results of neurotoxicity tests of C9 aromatic substances are shown in Table 3. The study of the complex C9 aromatic solvent was a subchronic study in which rats were exposed by inhalation at levels of 500, 2500 or 7500 mg/m^3, 6 hours/day, 5 days/week for 13 weeks. The rats were tested for effects on motor activity and in functional observation batteries after 30, 60 and 90 days of exposure and were then sacrificed to provide tissue for detailed pathological examinations of the nervous system. It should be noted that the animals were held without exposure for 48 hours prior to the motor activity and FOB testing to avoid potential confounding from acute effects. There was no evidence of persistent neurological effects at any of the exposure levels used in this study. In separate studies, it was found that a complex C9 aromatic solvent produced acute central nervous system (CNS) effects at a lower exposure level (200 mg/m^3) than the 1,2,4 TMB (1250 mg/m^3) tested under the same conditions. Thus, the evidence indicates that the complex C9

aromatic solvents are not less effective in producing acute CNS effects than the substances individually. In addition to these reports, there have been studies of the acute CNS effects of all three TMB isomers and one propylbenzene isomer, and repeated exposure studies of two of the TMB isomers as well as one propylbenzene isomers. All of the individual isomers produced acute CNS effects, but none produced persistent neurological effects. Thus, these data provide very consistent evidence that C9 aromatics can produce acute CNS effects, but that these effects are reversible and do not develop into chronic neurological conditions.

Representative complex C9 aromatic solvents have been tested for reproductive toxicity and found to have no effect on fertility. The individual isomers have not been specifically tested, but in 4 of the 5 individual isomer studies, the results of gross and pathological investigations indicated that the reproductive system was not a target for C9 aromatics (4-ethyltoluene being the exception), and in one study (cumene), spermatogenesis was assessed and found to be unaffected by treatment.

(3) Further hazard characterization tests are unnecessary as sufficient data are already available to address regulatory and public health needs.

With respect to the NTP proposal to test for repeated dose, developmental, neurotoxic and reproductive properties of C9 aromatic isomers, numerous studies of the isomers have already been conducted assessing most of these endpoints, and the data obtained does not indicate that the studies of representative mixed isomer substances failed to identify important toxicological effects or underestimated the toxic potency of these substances. We agree with the NTP that "inhalation exposure studies require extensive resources and it would be inefficient to conduct multiple separate studies for individual C9 alkylbenzenes and for multiple different endpoints". It is our view that because sufficient testing has already been conducted, resources would be better directed at making the best use of the data that is already available.

A review of the papers on individual C9 isomer substances provides evidence that the toxicological properties of these substances are very similar (which is expected as they have similar structures, similar physical and chemical properties and similar metabolic pathways). Across this range of substances, it is apparent that in animals, the principal effects of C9 aromatic hydrocarbons are acute central nervous system depression and upper respiratory tract irritation. The studies in animals of complex C9 aromatic hydrocarbon substances and individual isomers have provided limited evidence of systemic effects. Further, these substances are not mutagenic, do not produce selective developmental effects, do not affect fertility, and do not cause persistent neurological effects. Accordingly, further studies of these substances are unlikely to result in additional useful data.

(4) Further carcinogenesis tests specifically are unnecessary as the outcomes can be reasonably predicted from existing data, and, additionally, that the anticipated results would be difficult to interpret in a public health context.

As indicated by the NTP, there have been carcinogenicity studies of cumene and naphthalene. These substances are respiratory irritants, particularly in rodents, and chronic exposure resulted in tumors of the respiratory tract. The questions now at issue are whether these tumors are the consequence of persistent irritation and whether or not they are relevant to humans (e.g., Cruzan et al., 2009; Rhomberg et al., 2010). It is our belief that a better understanding of the processes by which respiratory irritants cause tumors in rodents would be of more help to regulatory and public health partners than the generation of more hazard characterization data.

II. RESPONSE TO SPECIFIC AIMS OF THE NTP PROPOSAL

a) ***Determine appropriate C9 alkylbenzene test agents for toxicity and carcinogenicity studies based on input from regulatory stakeholders*** – Per the detailed discussion provided above, we believe that individual C9 isomers have been extensively studied, that the hazards of these substances have already been well characterized, and that the available data support the view that it is more effective to test the C9 isomers collectively than individually. Further, in light of the irritating properties of these substances, we doubt that additional carcinogenicity tests would produce useful data.

b) ***Evaluate the toxicity and carcinogenicity of up to two test agents (as determined by Aim 1) following prechronic and chronic whole body inhalation exposure studies including reproductive, developmental, neurotoxicity endpoints*** – In our previous answer, we questioned whether any additional testing for hazard assessment would be useful. Beyond that, we believe that the reproductive, developmental, and neurotoxicological properties of these substances have been fully characterized and that no further testing for these endpoints can be justified.

c) ***Conduct short-term inhalation toxicity studies for additional C9 alkylbenzenes that are not included in Aim 2 designed to compare toxicity profiles between the various C9 isomers –*** We do not think that any further testing is necessary. That having been said, as the principal hazards of these substances are related to their potential to produce acute CNS effects and upper respiratory tract irritation, we believe that the best research strategy is to test these properties in acute *in vivo* toxicity tests.

d) ***Explore the feasibility of using novel in vitro systems for volatile chemicals to generate a targeted in vitro assessment of the C9 alkylbenzenes*** – The use of closed *in vitro* systems have been used for many years to test the genotoxic properties of gases and volatile

organic compounds. It is not clear what other innovations might be needed to test the C9 aromatics. However, more importantly, it is not clear why *in vitro* testing is even being considered for these substances. As the only clear effects are to be related to the potential to produce acute CNS effects and respiratory irritation, it would seem more efficient to simply conduct acute inhalation toxicity studies than to develop *in vitro* assays.

CONCLUSION

In conclusion, the ACC Hydrocarbon Solvents Panel believes that sufficient information is already available on the toxicological hazards of C9 aromatic molecules and the complex substances in which they are found, and that no further testing of subchronic toxicity, developmental toxicity, reproductive toxicity or neurotoxicity is justified. We believe that the potential for carcinogenic effects can be reasonably predicted from the existing data, and that resources would be better used in understanding the implications of results of carcinogenicity studies of similar substances than in the generation of more hazard characterization data. Finally, the proposal to develop *in vitro* tests is difficult to understand as the objectives are undefined, but as we consider the hazards of these substances to have already been well characterized, we do not believe that further *in vitro* work could be justified.

If you have any questions regarding these comments, please contact the Manager of the ACC Hydrocarbon Solvents Panel, Jon Busch, at jon_busch@americanchemistry.com; 202 249-6729).

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Table 1.
Repeated Dose Effects – Complex C9 Aromatic Solvent vs. 5/8 C9 Isomers

	Complex Solvent[a]	TMB 1,2,3	TMB 1,2,4	TMB 1,3,5	4 ET	Cumene (Isopropyl Benzene)
Concentration	1800, 3700, 7400[b]	123, 492, 1230	129, 492, 1207	50, 200, 600[c]	500, 1500, 5000	500, 2500, 6000
Deaths	None	None	None	None	None	None
Terminal BW	↓3700, 7400	No significant difference	No significant difference	No significant difference	Not reported	No significant difference
Organ Weight	↑Liver, kidney (3700, 7400)	↑Liver wt.	No significant difference	↑Liver, kidney (600)	↑Liver (5000)	↓Liver, kidney (6000)
Pathology	No findings	No findings	No findings	No findings	No findings	Kidney changes (male rats)
Hematology[d]	↓RBC (female)	↓RBC (female)	↓RBC (female)	None	None	None
Clinical Chemistry[e]	↑ALP (male, 7400)	No significant difference	No significant difference	↑ALP (600)	No significant difference	No significant difference
Testicular Effects	None	None	None	Not reported	↓Weight (5000)	None
NOAEC	1800 mg/m^3	492 mg/m^3	492 mg/m^3	600 mg/kg	1500 mg/m^3	500 mg/m^3

[a]Substance contained 47% TMB isomers, 30% ET isomers, 5% propyl benzene isomers, 5% xylene.
[b]All exposure levels given in mg/m^3.
[c]Oral administration, doses given in mg/kg.
[d]Red blood cell effects.
[e]Liver enzyme markers.

Table 2.
Comparison of Developmental Toxicity Studies of Complex C9 Aromatic Solvents and C9 Aromatic Constituents

Complex C9	Complex C9 Aromatic Solvent[a,b]	1,2,4 TMB	1,3,5 TMB	4 ET[c]	Cumene
Exposure Levels[d]	500, 2500, 7500	500, 1500, 3000, 4500	500, 1500, 3000, 6000	25, 100, 200 mg/kg	500, 2500, 6000
Deaths	14/32 (7500)	None	None	None	None
Maternal BW	↓(2500, 7500)	↓(4500)	↓(1500, 3000, 6000)	NSD[e]	NSD
Corpora lutea	NSD	NSD	NSD	NSD	NSD
Implantation loss	NSD	NSD	NSD	NSD	NSD
Dead fetuses	↓(7500)	NSD	NSD	NSD	NSD
Fetal BW	↓(2500, 7500)	↓(3000, 4500)	↓(3000, 6000)	NSD	NSD
Malformations	↑(7500)[f]	NSD	NSD	NSD	NSD
Variations	↑(7500)[g]	NSD	NSD	NSD	NSD
Maternal NOAEC (L)	500	1500 mg/m^3	500 mg/m^3	200 mg/kg[h]	2500 mg/m^3
Fetal NOAEC (L)	500	1500 mg/m^3	1500 mg/m^3	200 mg/kg	6000 mg/m^3

[a]Complex C9 aromatic solvent – 54% TMB, 27% ET, 7% propyl benzene, 6% C10 alkyl benzenes, 3% xylene.
[b]Complex C9 aromatic solvent study in mice.
[c]4-ET study by oral administration.
[d]Exposure levels given in mg/m^3 unless otherwise indicated.
[e]NSD = no significant difference.
[f]Increase in cleft palate (a stress response in mice) at high exposure level.
[g]Delayed development at high exposure level.
[h]200 mg/kg ≈ 800 mg/m^3.

Table 3.
Assessment of Neurotoxic Potential of Complex C9 Aromatic Solvents and C9 Aromatic Constituents

Substance	C9 Aromatics[a]	C9 Aromatics[b]	1,2,3 TMB[c]	1,2,4 TMB[c]	1,3,5 TMB[c]	1,2,4 TMB[d]	1,3,5 TMB[e]	Cumene[f]
Acute Neurotoxicity	Not assessed	8 hours exposure 200, 1000, 5000 mg/m^3 LOAEC = 1000 mg/m^3 NOAEC = 200 mg/m^3	4 hours exposure 1250-10000 mg/m^3 NOAEC = 2500 mg/m^3	4 hours exposure 1250-10000 mg/m^3 NOAEC = 2500 mg/m^3	4 hours exposure 1250-10000 mg/m^3 NOAEC = 2500 mg/m^3	Not assessed	Not assessed	6 hours exposure 500-6000 mg/m^3 LOAEC = 2500 mg/m^3 NOAEC = 500 mg/m^3
Subchronic Neurotoxicity (Persistent Effects)	90 day exposure 500, 2500, 7500 mg/m^3	Not assessed	Not assessed	Not assessed	Not assessed	4 weeks exposure 125, 500, 1250 mg/m^3	4 weeks exposure 125, 500, 1250 mg/m^3	13 weeks exposure 250, 500, 2500, 6000 mg/m^3
(A) Motor Activity	NSD	Not tested	Not assessed	Not assessed	Not assessed	Not assessed	Not assessed	No reproducible effects
(B) Functional Observation	NSD	Not tested	Not assessed	Not assessed	Not assessed	Not assessed	Not assessed	NSD
(C) Neuopathology	No pathologic changes	Not tested	Not assessed	Not assessed	Not assessed	Not assessed	Not assessed	No pathologic changes
(D) Auditory Function	Not assessed	Not assessed	Not assessed	Not assessed	Not assessed	Not assessed	Not assessed	NSD
Radial Maze	Not tested	Not tested	Not assessed	Not assessed	Not assessed	NSD	NSD	Not assessed
Open Field Activity	Not tested	Not tested	Not assessed	Not assessed	Not assessed	NSD	NSD	Not assessed
Passive Avoidance	Not tested	Not tested	Not assessed	Not assessed	Not assessed	NSD	NSD	Not assessed
Active Avoidance	Not tested	Not tested	Not assessed	Not assessed	Not assessed	NSD	NSD	Not assessed
Hot Plate Test	NSD	Not tested	Not assessed	Not assessed	Not assessed	NSD	NSD	Not assessed

[a]Douglas, J. et al. (1993), Toxicology and Industrial Health 19:1047-1058.
[b]McKee, R. et al. (2010), International Journal of Toxicology 29:277-290.
[c]Korsak, Z. and Rydzynski, K. (1996), International Journal of Occup. Med. Env. Health 9:341-349.
[d]Gralewicz, S. et al. (1997), Neurotoxicology and Teratology 19:327-333.
[e]Wiaderna, D. et al. (2002), International Journal of Occup. Med. Env. Health 15:385-391.
[f]Cushman, J. et al. (1995) Journal Am. College Toxicol. 14:129-147.

APPENDIX

Detailed summaries of the existing data are provided below:

(a) Repeated dose studies – The papers by Korsak et al. (2000a;b) describe similar studies in which rats were exposed to the test materials by inhalation, 6 hours/day, 5 days/week for 3 months. Exposure levels were 123, 492, and 1230 mg/m^3. The animals were observed during the exposure period and then examined for clinical, hematological and pathological effects at study termination. In both of the studies all of the rats survived the treatment period and there were no differences in terminal body weights. There was also an increase in sorbitol dehydrogenase in both studies. In the study of 1,2,3 TMB, there was an increase in liver weights in the high exposure group and a reduction in red blood cell counts, but no pathological changes were reported. In the study of 1,2,4 TMB there was a reduction in red blood cell count and an increase in white blood cell count, but no organ weight differences and no pathological changes. In the cumene study in which rats were exposed at levels of 50 ppm, 100 ppm, 500 ppm or 1200 ppm (approximately 250, 500, 2500 or 6000 mg/m^3), there were increases in some organ weights and evidence of male rat nephropathy in rats exposed to 500 or 1200 ppm, but no pathological changes other than those in the kidneys of male rats. The study of 4-ethyltoluene is less well described, but in the absence of any reported effects other than those associated with respiratory effects, it would be reasonable to assume that there were no notable systemic effects. In summary, there have been repeated dose studies of a complex aromatic substance, as well as studies of 2 TMB isomers, one ET isomer and cumene. These studies indicate that the systemic effects of repeated exposure are limited to some small and reversible hematological effects and increased organ weights without pathological changes. Recovery studies indicate that these effects are reversible. There is no evidence to suggest that the effects of the C9 isomers individually differ from those of the mixed isomer substance.

(b) Developmental Toxicity – The first reports of developmental toxicity studies of complex C9 aromatic solvents were from Ungvary et al. (1983) in which rats were exposed approximately 24 hours/day to vapors of "Aromatol" (a commercial solvent that contained 48% trimethylbenzene isomers, 38% ethyltoluene isomers and 9% propylbenzene isomers) at concentrations of 600, 1000, or 2000 mg/m^3 on gestational days 6-15. The rats were sacrificed on gestational day 21 and the uterine contents were examined. The authors reported that there was no evidence of fetal mortality or major malformations in these animals but that there were developmental delays (reduced fetal body weights, increased skeletal anomalies) in the 1000 and 2000 mg/m^3 exposure groups. The authors reported that there were also significant reductions in maternal body weight gain at these exposure levels. In a subsequent report, Ungvary and Tatri (1985) reported similar results from studies in mice and rabbits.

McKee et al. (1990) reported a study in which mice were exposed 6 hours/day to 500, 2500 or 7500 mg/m^3, 6 hours/day on days 6-15 of gestation. At the highest concentration, 14/32 of the

dams died, and there was a significant reduction in fetal body weights, and an increase in cleft palates (a stress effect in mice). In the 2500 mg/m^3 group, there were two maternal deaths, and a significant reduction in fetal body weight. In the low exposure group (500 mg/m^3), there were no treatment-related effects.

Saillenfait et al. (2005) reported that in developmental toxicity studies of 1,2,4 and 1,3,5-TMB, there was no evidence of embryolethal or teratogenic effects in rats. There was evidence of significant reductions in fetal weights but only at levels that also caused maternal effects. The no effect levels for maternal effects were in the range of 500-1500 mg/m^3 and the no effect levels for fetal effects were 1500 mg/m^3.

In studies of developmental effects of cumene in rats and rabbits, Darmer et al. (1997) reported that there were no treatment-related increases in incidences of external visceral or skeletal malformations or in the incidences of variations at any level. In rats the no effect level for maternal toxicity was 500 mg/m^3 and the no effect level for developmental toxicity was 6000 mg/m^3. In rabbits maternal toxicity was observed at the lowest concentration tested (1500 mg/m^3) but there were no developmental effects at the highest level tested (> 11,000 mg/m^3). In summary there have been studies of mixed C9 aromatic solvents from two independent laboratories as well as studies of 3 individual C9 isomers. Three species (rat, mouse, rabbit) were tested. The only effects were developmental delays at levels > 500 mg/m^3 and only in the presence of maternal toxicity.

(c) Neurotoxicity – In the first published studies (Douglas et al., 1993), rats were exposed 6 hours/day, 5 days/week for 13 weeks to a mixed C9 aromatic solvent at levels of 500, 2500 or 7500 mg/m^3. The rats were tested for motor activity and in a functional observation battery after 30, 60 and 90 days of exposure. At termination, the rats were sacrificed for a detailed pathological examination of the nervous system. There were no signs of neurotoxicity in any of the evaluated parameters and no evidence of pathologic changes in the nervous system. In reference to some of the later studies it should be noted that the animals were held without exposure for 48 hours before the motor activity and FOB examinations to avoid complications from acute CNS effects.

In 1996, Korsak and Rydzynski reported a study in which rats were exposed for 4 hours to each of the individual TMB isomers at levels ranging from 1250 – 10000 mg/m^3 and then assessed for acute CNS effects using rotarod performance and pain sensitivity (time to paw-lick on a hot plate). In the hot plate test which was conducted immediately after exposure, time to response was reduced at exposure levels greater than approximately 2000 mg/m^3. In a subsequent study rats were exposed for 3 months to 1,2,3- or 1,2,4-TMB at levels of 125, 500, or 1250 and then assessed for latency to paw-lick. The latency was significantly increased at exposure levels > 100 ppm; however, there were no differences between treated and control animals when the assessment was made two weeks after exposure. In this respect the Korsak and Rydzynski and Douglas studies are

consistent, exposure to the TMB isomers either individually or collectively increases latency to response in the hot plate test when the assessment is done immediately after exposure, but no effects were observed in assessments conducted either 2 days or two weeks after exposure. The studies by Gralewicz et al. (1997), Gralewicz and Wiaderna (2001) and Wiaderna et al. (2002) provide further evidence that trimethylbenzenes produce acute but not persistent effects on the central nervous system. In these studies rats were exposed to 1,2,3 TMB (Gralewicz and Wiaderna, 2001), 1,2,4 TMB (Gralewicz et al., 1997, Gralewicz and Wiaderna, 2001) or 1,3,5 TMB (Gralewicz and Wiaderna, 2001; Wiaderna et al., 2002). In each of these studies rats were exposed to the individual TMB isomers for 4 weeks (6 hours/day, 5 days/week) at various exposure levels. The animals were then held for at least 2 weeks without exposure, and then tested for persistent neurological effects using a battery of tests including radial maze, open-field activity, and hot plate. There were few differences in any of these tests providing additional evidence that the neurological effects were acute but not persistent.

McKee et al. (2010) reported a study in which the acute CNS effects of 1,2,4 TMB and a complex C9 aromatic solvent were assessed. In these studies rats were exposed to vapors at levels ranging from approximately 100 to 5000 mg/m^3, 8 hours/day for 3 consecutive days. The potential for acute CNS effects was assessed immediately after the first and third exposures and again 24 hours after the last exposure period. There were effects on gait and motor activity in high exposure group animals tested immediately after exposure. There was also an increase in latency to respond in high dose group animals in a visual discrimination test. However, there were no effects in animals tested 24 hours after exposure. The overall effect levels were approximately 5000 mg/m^3 and the no effect levels were > 1000 mg/m^3.

Cushman et al. (1995) reported effects on gait in rats exposed to cumene at levels of approximately 2500 and 6000 mg/m^3 for 13 weeks. The effects were most pronounced at 1 hour and largely reversed at 6 hours. Repeated exposures did not result in any functional or pathological changes in the central nervous system.

In summary, studies of the neurological effects of complex C9 aromatic solvents, the three TMB isomers tested separately, and cumene, indicated that these substances could cause acute CNS effects at levels, in most cases at levels > 1000 mg/m^3. However, there were no persistent functional effects and no pathological changes.