

May 16, 2003

Dr. Mary S. Wolfe
NTP Executive Secretary
PO Box 12233
111 T.W. Alexander Dr.
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Research Triangle Park, NC 27709

Re: Comments on Draft TR-519, Stoddard Solvent Type IIC

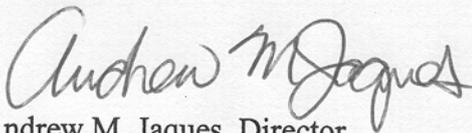
Dear Dr. Wolfe:

The American Chemistry Council Hydrocarbon Solvents Panel (the "Panel") is pleased to provide the attached comments on draft TR-519, Stoddard Solvent Type IIC, for consideration by the National Toxicology Program (NTP) Board of Scientific Counselors Technical Reports Review Subcommittee. The Panel represents manufacturers of hydrocarbon solvents, including Stoddard Solvent, Type IIC.

As I mentioned in my previous letter, Arlean Medeiros of ExxonMobil Biomedical Sciences, Chair of the Panel's Toxicology Research Task Group, will be presenting these comments to the Subcommittee at its May 22 meeting.

Please contact me if you have any questions. I can be reached at 703-741-5627 or by email at Andrew_Jaques@americanchemistry.com.

Warmest Regards,



Andrew M. Jaques, Director
Hydrocarbon Solvents Panel



**American Chemistry Council
Hydrocarbon Solvents Panel**

**Comments to the National Toxicology Program Board of Scientific Counselor's
Technical Report Review Subcommittee on Draft Technical Report on the Toxicology
and Carcinogenesis Studies of Stoddard Solvent IIC in F344/N Rats and B6C3F₁ Mice
(TR-519)**

Introduction

The Hydrocarbon Solvents Panel¹ (the "Panel") appreciates the NTP's efforts in conducting and presenting these toxicology tests and welcomes the opportunity to comment on the draft Technical Report on the Toxicology and Carcinogenesis Studies of Stoddard Solvent IIC in F344/N Rats and B6C3F₁ Mice (TR-519). The Panel represents manufacturers of hydrocarbon solvents, including Stoddard Solvent IIC. The Panel is preparing toxicology assessments of hydrocarbon solvents as a part of its sponsorship of this substance and a number of other hydrocarbon solvents under the International Council of Chemical Associations (ICCA) High Production Volume (HPV) Chemical Initiative. The ICCA initiative includes the development of Screening Information Data Sets (SIDS) dossiers and SIDS Initial Assessment Reports and the presentation of this information to the Organization for Economic Cooperation and Development (OECD). These assessments are ongoing, although preliminary information relevant to Stoddard Solvent IIC is available and has been shared with NTP. Overall, the toxicology assessment based on the compiled data is consistent with the draft NTP TR-519. These data show that those hydrocarbon solvents which could be generically described as "Stoddard Solvent" have a low order of acute, repeated dose, and reproductive/developmental toxicity by the oral, dermal, and inhalation routes of exposure. *In vitro* and *in vivo* genotoxicity assays show no evidence of mutagenic or genotoxic activity. The most common effects observed in repeated dose toxicity studies are histological changes consistent with α_{2u} -globulin-mediated renal effect in male rats. Because α_{2u} -globulin is not present in humans, the US EPA has determined that effects associated with α_{2u} -globulin induction in the male rat kidney are not useful for assessing human risk (US EPA, 1991).

¹ Members of the Hydrocarbon Solvents Panel are CITGO Petroleum, ExxonMobil Chemical Company, Flint Hills Resources LP, Sasol North America, Inc., Shell Chemical LP, and Sunoco, Inc.

The Panel believes the draft NTP TR-519 is generally well-written and thorough. However, the Panel believes that the induction of pheochromocytoma in the male rats was a secondary effect related to kidney toxicity. Thus, the Panel has reservations about the proposed conclusion “some evidence of carcinogenic activity” in male rats and believes that the NTP should more thoroughly address the relationship of chronic nephropathy and α_{2u} -globulin mediated nephropathy to the induction of these tumors and consider an alternate conclusion “equivocal evidence of carcinogenic activity”. Additionally, the change in the NTP rat diet may also have introduced a confounding factor, and its possible relationship to fluctuations in the underlying spontaneous tumor response should be examined.

The Panel also requests that the NTP change page 35 and Appendix J, Chemical Characterization and Generation of Chamber Concentrations, to state that the test material was provided by a member of the American Chemistry Council Hydrocarbon Solvents Panel and not specifically the Shell Chemical Company. This test material is representative of the class of hydrocarbon solvents conforming to ASTM D-235, Type IIC, and the Panel requests that it not be associated with a specific manufacturer, commercial product, or trade name.

Evaluation of the Pheochromocytomas and the Relationship to Nephropathy in Male F/344 Rats

The Panel has carefully evaluated the pathology data provided on the NTP website for this study and also obtained an independent review of these data by Dr. E. Eugene McConnell, DVM, MS, DABT. In reviewing these data, the Panel agrees with the NTP’s selection of benign pheochromocytomas in male rats as the major neoplastic finding on a statistical basis. However, in further reviewing these data, the Panel believes that there is strong evidence that the increase in the benign pheochromocytomas was a secondary effect related to renal disease (nephropathy), mediated by the increase in α_{2u} -globulin, and not a direct effect of exposure to Stoddard Solvent IIC. The Panel bases this conclusion on the following points:

1. The increased incidence of total pheochromocytomas (benign and malignant) and nephropathy were seen only in male rats, along with a documented increase in α_{2u} -globulin nephropathy in male rats.
2. The pheochromocytomas were benign and did not progress to malignancy.
3. The increased incidence of benign pheochromocytomas appears to have been associated with increased severity of nephropathy in male rats at the high dose.
4. There was no increase in the severity of nephropathy in female rats, and, consistent with theory, the frequency of pheochromocytomas was similarly not increased.
5. There is a plausible mode of action relating renal nephropathy to induction of pheochromocytoma. Experimental data indicating that the renal effects of Stoddard Solvent IIC, particularly induction of α_{2u} -globulin nephropathy in male rats, are consistent with that mode of action and provide a plausible explanation for the increased frequency of pheochromocytomas in the male rats.

1. Male Rat Specific Effect

In considering the relationship between adrenal pheochromocytomas and nephropathy it is important to note that the increase in pheochromocytomas was only seen in male rats. If Stoddard Solvent IIC caused pheochromocytomas directly, a similar response in the adrenal medulla of female rats would be expected, at the very least there should have been a hyperplastic response. However, no responses of this type were observed in female rats. This male rat-specific response further supports the plausibility of an indirect α_{2u} -globulin-mediated response to the adrenal medulla.

As seen with other similar hydrocarbon solvents, Stoddard Solvent IIC causes α_{2u} -globulin nephropathy. The most significant exposure-related pathologic findings in rats were the spectrum of renal lesions in males, characteristic of renal toxicity related to chronic accumulation of α_{2u} -globulin (page 89, Table 11). Additional evidence demonstrating that the nephropathy was related to α_{2u} -globulin and not Stoddard Solvent

IIC *per se* is the lack of renal toxicity in female rats, in spite of the fact that the high dose in female rats was twice that for male rats.

2. *No Increase in Malignant Pheochromocytomas*

If these benign neoplasms were a direct effect of the chemical exposure, progression to a malignant state would be expected. In this case, no statistically significant increase in malignant pheochromocytomas was observed.

3. *Incidence of Pheochromocytoma Related to Severity of Nephropathy in Male Rats*

A possible correlation between severe nephropathy and pheochromocytoma in male F344 rats in the NTP database was reported by Nyska et al. (1999). In rats surviving beyond 21 months, the incidence of adrenal pheochromocytoma was consistently higher in animals with more severe chronic nephropathy, $p < 0.05$, for both 900 NTP inhalation study controls and 900 NTP feeding study controls (Nyska et al. 1999).

For Stoddard Solvent IIC, the severity of nephropathy appears to be significantly greater in high dose male rats with pheochromocytomas than in rats without the tumor. The average nephropathy score of high dose male rats with pheochromocytomas was 3.05 compared to 2.61 for high dose male rats without pheochromocytomas. Attachment A shows a statistical analysis of the relationship between dose, sex, nephropathy severity, and incidence of pheochromocytoma. These analyses show that there is a relationship ($p = \sim 0.05 - 0.10$) between nephropathy severity and incidence of pheochromocytoma in the high dose males. When males from all dose groups were combined, the statistical analysis of the nephropathy score was not related to the presence of pheochromocytomas, but it was statistically correlated to dose group ($p = 0.03$) and survivorship ($p = 0.04$). That is, the higher dose and longer survivorship were positively related to the presence of pheochromocytomas. These findings are consistent with results described by Nyska *et al.* (1999) and provide evidence for the dose- and time-dependent progression of α_{2u} -globulin nephropathy in male rats (US EPA, 1991).

4. *No Increase in Nephropathy Severity or Pheochromocytomas in Female Rats*

There is no increase in the severity of nephropathy or incidence of pheochromocytomas in female rats. This effect appears to be present only in one sex and

one species, male rats. A statistical analysis for female rats showed that the nephropathy score was also related to presence of benign pheochromocytoma ($p = 0.03$). So, these data were consistent with the hypothesis of a link between renal effects and pheochromocytoma induction even though renal changes were not associated with Stoddard Solvent administration.

5. *Proposed Mode of Action of Pheochromocytomas Relates to α_{2u} -globlin Nephropathy in Male Rats*

The pathogenesis of pheochromocytomas in rats can include genetic background, chronic high levels of growth hormone or prolactin associated with pituitary tumors, dietary factors, and stimulation of the autonomic nervous system (Rosol *et al.*, 2001). Tischler *et al.* (1995) showed that chronic administration of reserpine, a hypertensive drug, stimulated the chromaffin cells in the adrenal medulla, thus resulting in increased cell proliferation. Later work by Tischler *et al.* (1996, 1999) using vitamin D₃, a potent chromaffin cell proliferator, showed that the nature and intensity of the neurally derived signals that stimulate chromaffin cell proliferation may be related to the mitogenic effects of Ca²⁺ homeostasis. Chromaffin cells utilize Ca²⁺ from both extracellular fluid and intracellular stores as a second messenger for regulating numerous cell functions. In addition to receptor and voltage-gated Ca²⁺ channels, in rat chromaffin cells, a distinctive characteristic is the presence of spontaneous oscillation in cytosolic Ca²⁺, a pacemaker activity that could render rat chromaffin cells particularly sensitive to altered Ca²⁺ homeostasis (Tischler *et al.* 1996). Chronic renal failure could also result in low serum calcium levels thereby stimulating parathyroid hormone secretion (Nyska *et al.*, 1999). These studies show that perturbations in Ca²⁺ homeostasis can lead to increased chromaffin cell proliferation and pheochromocytomas, with particular sensitivity in rats.

The proposed mode of action that Stoddard Solvent IIC induces pheochromocytomas in male rats relates to α_{2u} -globlin nephropathy. Stoddard Solvent IIC, as seen with other hydrocarbon solvents, causes α_{2u} -globlin nephropathy in male rats, a widely accepted male rat-specific effect with no relevance to human risk assessments (US EPA, 1991). We propose that in male rats, as a result of α_{2u} -globlin, a cascade of renal effects occur, of which the primary effect exacerbates chronic nephropathy, leading to perturbations in the Ca²⁺ homeostasis. This results in secondary parahyperthyroidism and

hypercalcemia. Secondary hyperthyroidism, associated with chronic renal disease, is believed to occur due to reduced filtration of the glomeruli, leading to the retention of phosphorus, resulting in hyperphosphatemia and decreased blood calcium levels (Kremer *et al.* 1989). The association between hypercalcemia and increased incidence of pheochromocytomas in rats has been previously reported in rats treated with retinol acetate for 2 years (Kurokawa *et al.*, 1985).

The proposed mechanism for Stoddard Solvent IIC is that induction of α_{2u} -globulin nephropathy in male rats results in hypercalcemia. This, in turn, stimulates the adrenal medulla, increasing the rate of cell proliferation and ultimately producing adrenal pheochromocytomas. This mechanism is consistent with evidence that Stoddard Solvent IIC is not mutagenic or genotoxic *in vitro* and *in vivo* test systems, and, therefore, induces these tumors indirectly, via a non-genotoxic process.

Decalin, decahydronaphthalene, another solvent that was recently tested by the NTP (NTP TR-513), also caused an increase in male rat nephropathy and a male-only increase in adrenal pheochromocytomas. The incidences of these pheochromocytomas in male rats in the Decalin study are similar to the findings in Stoddard Solvent IIC study (see table below), and both substances induced α_{2u} -globulin mediated nephropathy.

Incidences of Pheochromocytomas in Male Rats - Decalin
(NTP TR-513)

	% Incidence (no. of animals/total animals)				
	Control	25 ppm	50 ppm	100 ppm	400 ppm
Pheochromocytoma, benign	14 (7/49)	18 (9/49)	22 (11/49)	20 (10/49)	30 (6/20)
Pheochromocytoma, malignant	4 (2/49)	0 (0/49)	4 (2/49)	14 (7/49)	15 (3/20)
Pheochromocytoma, benign and malignant	16 (8/49)	18 (9/49)	27 (13/49)	33 (16/49)	40 (8/20)

Source: NTP TR-513, Table 13, page 69.

Incidences of Pheochromocytomas in Male Rats - Stoddard Solvent IIC
(NTP TR-519)

	% Incidence (no. of animals/total animals)			
	Control	138 mg/m ³	550 mg/m ³	1,100 mg/m ³
Pheochromocytoma, benign	10 (5/50)	18 (9/50)	26 (13/50)	34 (17/50)
Pheochromocytoma, malignant	1	0	0	2
Pheochromocytoma, benign and malignant	12 (6/50)	18 (9/50)	26 (13/50)	38 (19/50)

Source: NTP TR-519, Table 9, page 68.

NTP Rat Diet

The Panel asks the NTP to consider the impact of changes to the NTP rat diet on the results and interpretation of the findings of this Stoddard Solvent IIC study. While these results may not be a spurious (by chance) finding, the marked difference in the historical incidence using the “new” NTP diet (NTP-2000) interjects a confounder that should be considered and mentioned in the technical report. As noted in the table below, the historical incidence of pheochromocytomas in rats fed NTP-2000 diet decreased by about 50% compared to that observed with the “old” NTP diet (NIH-07). In fact, the incidence of pheochromocytomas found in the Stoddard Solvent IIC study in the high dose group (34%) is close to the control incidence in studies using the “old” diet (31.6%) (Source: NTP website). It should be noted that the historical control data were derived from studies conducted in the same laboratory (Battelle-NW), so interlaboratory variability would not explain the difference. The decreased pheochromocytoma incidence in controls appears to be related to the change in diet. As reported by Rao (1997), the effects of changing to the NTP-2000 diet were not evaluated in a 2-year bioassay; instead NTP-2000 and NIH -07 diets were evaluated in a 13-week study. This comparison showed that the NTP-2000, containing lower protein, higher fat and/or fiber resulted in lower adrenal weights, which may be related to a potential delay in early changes of medullary hyperplasia, leading to pheochromocytomas. However, a 13-week study would not be

sufficient to evaluate the incidence of pheochromocytomas, a tumor that is commonly found in aging rats (Tischler *et al.* 1995, 1999). Therefore, the effect of the NTP-2000 rat diet on the incidence of pheochromocytomas in chronic studies is unknown.

Incidence of Pheochromocytomas in Control Male and Female F344N Rats
on “old” and “new” NTP Diets

	Males (%)		Females (%)	
	Average	Range	Average	Range
Old diet, inhalation studies	31.6 (285/901)	8 - 50	5.3 (47/889)	0 - 10
New diet, inhalation studies	16.1 (48/298)	10 - 28	4.4 (13/296)	2 - 6
New diet, all studies	13.8 (145/1053)	5 - 28	4.3 (47/1098)	0 - 8

Source: NTP website

Source of Test Material

The Panel requests that NTP change the description on page 35 and in Appendix J, Chemical Characterization and Generation of Chamber Concentrations, to state that the test material was provided by a member of the American Chemistry Council Hydrocarbon Solvents Panel and not specifically the Shell Chemical Company. This test material is representative of the class of hydrocarbon solvents conforming to ASTM D-235, Type IIC, and the Panel requests that it not be associated with a specific manufacturer, commercial product, or trade name.

Conclusions

The increased incidence in adrenal pheochromocytomas that was only seen in male rats may have been secondary to chronic nephropathy. Given the evidence that these tumors are correlated with chronic nephropathy, the likelihood that these tumors were a secondary effect of α_{2u} -globulin nephropathy and their lack of relevance to humans should be discussed in the technical report.

The impact of the change in the rat diet should be addressed to identify the possible relationship to fluctuations in the underlying spontaneous tumorigenesis.

The Panel is considering pursuing additional information to support this mode of action of the formation of pheochromocytomas under influence of α_{2u} -globulin nephropathy.

References

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Attachment A

Statistical Analysis of Relationship Between Adrenal Pheochromocytomas and Nephropathy Scores in Rats - Stoddard Solvent IIC (NTP TR-519)

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Introduction

The statistical analyses were conducted to assess the relationship between adrenal pheochromocytomas and nephropathy scores in rats. Data were obtained from the Stoddard Solvent IIC Pathology data tables provided on the NTP website. These analyses considered the relationship among five measures on an animal.

- Presence/absence of a pheochromocytoma (dependent variable),
- Nephropathy score (scale 1 to 4)
- Dose group (4 groups including control)
- Survivorship (days on test)
- Sex of animal (M/F)

Discussion

Since there were a greater percentage of pheochromocytomas in the males (47 of 199 examined) than in the females (11 of 176), a 4-fold proportional increase, statistical analyses were conducted on each sex separately.

Using logistic regression which assumes that the predicted variable has a binary response (presence/absence of tumor), and the independent (predicting) variables were the dose group, survivorship and nephropathy score the calculated p values associated with the model are presented below.

Male Rats

An initial statistical analysis using chi square and t-tests was conducted in the high dose male rats. This showed a positive relationship of nephropathy score and the presence of adrenal pheochromocytoma, $p = 0.05 - 0.10$. This suggests that the higher nephropathy scores were associated with the presence of pheochromocytomas.

A series of analyses was conducted separately for each dose group to determine the statistical significance of the relationship between the presence/absence of a pheochromocytomas and nephropathy scores using the chi-square test. For these analyses, the relationship is statistically significant only in the high dose group ($p \sim 0.06$). The significance levels of the test in the control, low-dose and mid-dose groups were 0.46, 0.21, and 0.99 respectively. This indicates that the positive relationship seen in the high-dose group is not seen in the other dose groups.

Using a logistic regression model which included all dose groups, the analysis shows that the nephropathy score was not associated with tumor incidence in male rats (see table below). If the model is considered without dose and without survivorship, then there is a statistically significant relationship ($p \sim 0.09$). This explains the apparent contradiction of this analysis and the analysis with only the high-dose males which showed a statistically significant relationship ($p = 0.05 - 0.1$). In male rats, the dose group is a statistically stronger predictor of the tumor than is the nephropathy score – so when the analysis is conducted using only one dose group there is no influence from dose.

Significance Levels for Logistic Regression Model on Pheochromocytoma Incidence in Male Rats

Variable	p value
Survivorship	0.04
Dose group	0.03
Nephropathy score	0.63

Female Rats

In female rats, the nephropathy score is related to the presence of a pheochromocytoma ($p = 0.032$), while dose group and survivorship are not related.

Significance Levels for Logistic Regression Model on Pheochromocytoma Incidence in Female Rats

Variable	p value
Survivorship	0.78
Dose group	0.61
Nephropathy score	0.03

Other related statistical tests were also conducted to determine stability of these results among various statistical tests. All other tests support these results, therefore, these statistical analyses provide a reasonable representation of the statistical conclusions from these data.

Conclusions

The major conclusion is that the male and female rats have a different relationship between pheochromocytomas and nephropathy scores.

- In males, the nephropathy score is not related to the presence of pheochromocytomas, however, dose group and survivorship are related. This indicates that the higher dose and longer survivorship are positively related to the presence of the tumor.
- In females, the nephropathy score is related to the presence of a pheochromocytoma, i.e., the higher nephropathy score is related to the presence of the tumor, while dose group and survivorship are not related to the presence of a tumor.