Board of Scientific Counselors
National Toxicology Program

Summary Minutes
from

Peer Review of Draft Technical Reports of Long-Term
Toxicology and Carcinogenesis and Short-Term Toxicity Studies
by the Technical Reports Review Subcommittee

on

December 1-2, 1992

Research Triangle Park, North Carolina

The review meeting began at 9:00 a.m. on December 1 and 2 in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee are: Drs. Curtis Klaassen (Chairperson), Paul Bailey, Arnold Brown, Gary Carlson, Kowetha Davidson, Harold Davis, Daniel Longnecker, Louise Ryan, Ellen Silbergeld, Robert Taylor, Matthew van Zwieten, Jerrold Ward, Lauren Zeise, and Mr. Louis Beliczky. Drs. Longnecker, Silbergeld, and Zeise were unable to attend the meeting. These minutes have been reviewed and approved by all members of the Subcommittee who participated. They were written by Dr. Larry G. Hart, Executive Secretary.

When available, a limited number of final NTP Technical Reports for the studies may be obtained free of charge from the NTP Central Data Management, MD A0-01, P.O. Box 12233, Research Triangle Park, NC 27709. Telephone: 919/541-3419. Subsequently, they may be purchased from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, 703/487-4650.

The next NTP technical reports peer review meeting will be held June 22, 1993, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, 919/541-3971.

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SUMMARY MINUTES
TECHNICAL REPORTS REVIEW SUBCOMMITTEE MEETING
December 1-2, 1992

Benzyl Acetate. Dr. K.M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of benzyl acetate by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related non-neoplastic lesions in male and female mice. Benzyl acetate was studied previously by the NTP using the gavage route and corn oil as a vehicle. Because of the confounding effect of corn oil on the increased incidence of pancreatic neoplasms in male rats, the NTP decided to restudy the chemical using the dosed feed route. Dr. J. Yuan, NIEHS, reported on pharmacokinetic studies designed to compare the internal dose for the feed route compared with that for the gavage route. The pharmacokinetic studies demonstrated that blood levels of the major metabolite of benzyl acetate, benzoic acid, were up to 300 times greater after gavage administration than after administration in the feed. The conclusions for the current and previous studies were that:

> Under the conditions of these 2-year feed studies, there was **no evidence of carcinogenic activity** of benzyl acetate in male or female F344 rats receiving 3,000, 6,000, or 12,000 ppm. There was **no evidence of carcinogenic activity** of benzyl acetate in male or female $B6C3F_1$ mice receiving 330, 1,000, or 3,000 ppm.
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> Nasal lesions associated with benzyl acetate exposure in male and female mice included nasal mucosa atrophy and degeneration (primarily of the olfactory epithelium), cystic hyperplasia of the nasal submucosal gland, and luminal exudate and pigmentation of the nasal mucosal epithelium.
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> In previous 2-year gavage studies, benzyl acetate increased the incidence of acinar cell adenomas of the exocrine pancreas in male F344/N rats; the gavage vehicle may have been a contributing factor. There was **no evidence of carcinogenic activity** in female F344/N rats receiving 250 or 500 mg/kg per day. There was **some evidence of carcinogenic activity** in male and female $B6C3F_1$ mice, indicated by the increased incidences of hepatocellular adenomas and squamous cell neoplasms of the forestomach.

Dr. Davis, a principal reviewer, agreed in principle with the conclusions. He thought an MTD was not achieved in rats. Based on lack of mortality or clinical signs and only a modest effect on weight gain at 25,000 ppm in the 13-week studies, he suggested the top dose in 2-year studies should have been between 25,000 and 50, 000 ppm. Dr. Abdo said he agreed that rats could have tolerated a higher top dose but at the time the dose setting was done there was concern about reduced food consumption. Dr. Davis said that this kind of information needs to be put in the report.

Dr. Carlson, the second principal reviewer, agreed with the conclusions, although he agreed that the MTD was not reached in the rat studies. He asked whether it was true that nasal lesions did not occur in previous studies or whether they were

just not looked for. Dr. C. Shackelford, NIEHS, said there was no evidence of nasal lesions in the previous NTP study.

Dr. van Zwieten asked whether the rationale for doing this study included other than the corn oil effects noted in the first study. Dr. Abdo said the forestomach irritation as well as the fact that human exposure to benzyl acetate is more often to the skin or in food were also reasons. Dr. Davis asked for staff comment on the importance of reaching the MTD. Dr. G. Boorman, NIEHS, said it was quite important particularly when trying to compare effects in studies where the route or mode of chemical administration was different as here. Dr. Klaassen criticized the analytical method used for measuring blood levels of benzoic acid and also, wondered whether the toxicology of benzoic acid was being studied instead of benzyl acetate. Dr. T. Goehl, NIEHS, said the method was sensitive down to 1 μg/ml of benzoic acid and can also detect benzyl alcohol and hippuric acid; however, benzoic acid is the major component after either gavage or feed administration. Dr. B. Schwetz, NIEHS, said an important point in giving perspective to both studies is that the metabolism of benzyl acetate in rodents is probably quite similar to that in humans.

Dr. Davis moved that the Technical Report on benzyl acetate be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, **no evidence of carcinogenic activity**. Dr. Carlson seconded the motion and then offered an amendment that a statement be added to the conclusions that higher doses could have been tolerated in the 2-year rat studies. Mr. Beliczky seconded the amendment, which was accepted by six yes votes (Beliczky, Brown, Carlson, Davis, Taylor, Ward) to four no votes (Bailey, Davidson, Ryan, van Zwieten). The original motion by Dr. Davis as amended by Dr. Carlson was then accepted unanimously with ten votes.

o-Benzyl-p-Chlorophenol. Dr. D.S. Marsman, NIEHS, introduced the toxicology and carcinogenesis studies of o-benzyl-p-chlorophenol by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic lesions in female rats and male mice and non-neoplastic lesions in male and female rats and mice. The kidney was the primary target organ for toxicity in both species. Additional step-sections of the kidney were performed in male and female rats and mice. The conclusions for the studies were that:

> Under the conditions of these 2-year gavage studies, there was **no evidence of carcinogenic activity** of o-benzyl-p-chlorophenol in male F344 rats receiving 30, 60, or 120 mg/kg body weight. There was **equivocal evidence of carcinogenic activity** of o-benzyl-p-chlorophenol in female F344 rats based on the occurrence of two rare renal transitional cell carcinomas. There was **some evidence of carcinogenic activity** of o-benzyl-p-chlorophenol in male $B6C3F_1$ mice based on increased incidences of renal tubule adenoma and renal tubule adenoma or carcinoma (combined). There was **no evidence of carcinogenic activity** of o-benzyl-p-chlorophenol in female $B6C3F_1$ mice receiving 120, 240, or 480 mg/kg.
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> o-Benzyl-p-chlorophenol was nephrotoxic for male and female F344 rats and $B6C3F_1$ mice. The severity of nephrotoxicity was increased in rats of each sex and the incidence and severity of nephropathy was increased in mice of each sex. The incidence and severity of nephropathy increased with length of treatment. Other lesions considered to be associated with the nephropathy and the secondary hyperparathyroidism included fibrous osteodystrophy and soft tissue mineralization.

Mr. Beliczky, a principal reviewer, agreed with the conclusions. He questioned whether the testing conducted satisified concerns regarding consumer safety in household or hospital use. Mr. Beliczky asked if NIOSH could provide data for inclusion on the method of production and encountered health risks. Dr. J. Haartz, NIOSH, said the data cited are from the National Occupational Exposure Survey (NOES) and are potential not actual worker exposure.

Dr. van Zwieten, the second principal reviewer, agreed with the conclusions. He commented that since the rationale for the study included relationship chemically to a known neurotoxin, extra attention should have been given to morphological assessment of the central and peripheral nervous systems. Further, detailed procedures for the neurobehavioral tests should be provided. Dr. Marsman said the NTP standard neurobehavioral battery was used and more details could be included. He said that had there been any indication that the chemical was a neurotoxin, like hexachlorophene, additional pathology would have been included in the design.

Dr. Ward, the third principal reviewer, agreed in principle with the conclusions. He thought the dose-related increased incidences of renal tubular adenomas and carcinomas (combined) could support **clear evidence of carcinogenic activity** in male mice. He suggested that a sentence be added to the conclusions about the hyperplastic lesions of the mouse forestomach. Dr. Marsman said the severity of the hyperplasia did not increase in treated animals and was consistent with that

seen with chemicals given by gavage that are known to be irritants. Dr. Ward noted that though there was no depression of weight gain in rats, the renal lesions were severe enough to show that an MTD was reached.

Mr. Beliczky moved that the Technical Report on o-benzyl-p-chlorophenol be accepted with the revisions discussed and with the conclusions as written for male rats and female mice, **no evidence of carcinogenic activity**, for female rats, **equivocal evidence of carcinogenic activity**, and for male mice, **some evidence of carcinogenic activity**. Dr. van Zwieten seconded the motion, which was accepted unanimously with ten votes.

C.I. Direct Blue 218. Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of C.I. Direct Blue 218 by discussing the use and rationale for study (as part of the NTP Benzidine Dye Initiative), describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and non-neoplastic lesions in rats and mice. The conclusions were that:

> Under the conditions of these 2-year gavage studies, there was **some evidence of carcinogenic activity** of C.I. Direct Blue 218 in male F344 rats based on the occurrence of pharyngeal neoplasms. Squamous cell neoplasms of the forestomach may have been chemical related. There was **no evidence of carcinogenic activity** of C.I. Direct Blue 218 in female F344 rats given 1,000, 3,000, or 10,000 ppm. There was **clear evidence of carcinogenic activity** of C.I. Direct Blue 218 in male and female B6C3F$_1$ mice based on increased incidences of hepatocellular adenomas and carcinomas. The occurrence of a few neoplasms of the small intestine and kidney in male mice may have been related to C.I. Direct Blue 218 treatment.
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> The administration of C.I.Direct Blue 218 produced an increased incidence of forestomach basal cell hyperplasia in rats and hepatocellular foci of cytologic alteration in mice.

Dr. Davidson, a principal reviewer, agreed with the conclusions. She said the background information on the metabolism, toxicity, and carcinogenicity of the benzidine and benzidine-congener based dyes was detailed and appeared representative of the available literature.

Mr. Beliczky, the second principal reviewer, agreed with the conclusions. He said a more recent NIOSH Health Hazard Evaluation (HHE) also should be cited. Dr. J. Haartz, NIOSH, said this information would be provided if available.

Dr. Ward commented on the incidence of three kidney tumors in male mice at the lowest dose, wondering why this finding did not fall under **clear evidence** as these are rare tumors and there was a corresponding lack of renal toxicity. Dr. Dunnick responded that the lack of a dose response and the lack of statistical significance compared with controls led to the conclusions that these lesions might not be related to chemical treatment. Dr. Davis noted that the dermal route of exposure would more likely mimic workplace exposure than the oral route of administration. Dr. Ryan noted the teratogenic effects reported for other benzidine-based dyes and suggested reproductive and developmental toxicology studies would be appropriate. Dr. Dunnick reported that at the time the benzidine-based dye studies were initiated, potential for carcinogenesis was a primary concern but agreed that reproductive effects are of concern and are receiving increasing priority.

Dr. Davidson moved that the Technical Report on C.I. Direct Blue 218 be accepted with the revisions discussed and with the conclusions as written for male rats, **some evidence of carcinogenic activity,** for female rats, **no evidence of carcinogenic activity,** and for male and female mice, **clear evidence of carcinogenic activity**. Mr. Beliczky seconded the motion which was accepted unanimously with ten votes.

Corn Oil, Safflower Oil, and Tricaprylin (Comparative Toxicology Studies) Dr. G. A. Boorman, NIEHS, introduced the comparative toxicology studies of corn oil, safflower oil, and tricaprylin by reporting on the rationale for the studies. Corn oil has been used in the Program as the oil vehicle for gavage studies. NTP studies have shown that control male rats receiving a corn oil vehicle have a higher incidence of proliferative lesions of the exocrine pancreas and a lower incidence of mononuclear cell leukemia than untreated control males. The current NTP studies were designed to evaluate the role of several oils in altering cancer rates in male rats, and were part of a larger program that included cooperative agreements with Dartmouth Medical School, Northwestern Medical School, and the University of Missouri to evaluate potential mechanisms of corn oil in the induction of pancreatic cancer. To evaluate the potential role of corn oil in promoting a pancreatic proliferative effect, a parallel study was performed in which dichloromethane in corn oil was administered to groups of animals. Dr. Boorman described the experimental design, reported on survival and body weight effects, and commented on compound-related neoplastic and nonneoplastic lesions in male F344 rats. The results were summarized as:

> These studies demonstrate that safflower oil and tricaprylin do not offer significant advantages over corn oil as a gavage vehicle in long-term rodent studies. Both safflower oil, which is a polyunsaturated oil like corn oil but with markedly different fatty acid composition, and tricaprylin, which is a saturated medium-chain triglyceride, caused proliferative lesions of the exocrine pancreas and decreased incidences of mononuclear cell leukemia in male F344 rats. Further, corn oil used as a gavage vehicle may have a confounding effect on the interpretation of chemical-induced proliferative lesions of the exocrine pancreas.

Dr. van Zwieten, a principal reviewer, said the rationale for the studies was clear, and the studies were designed and conducted properly. Since the pancreas was a known target tissue, he thought it would be useful to provide additional descriptive information regarding diagnostic criteria for distinguishing pancreatic acinar cell hyperplasias from adenomas and carcinomas. Dr. Boorman agreed. Dr. van Zwieten said brief comments on the possible mechanism for the dose-related decrease in incidence of mononuclear cell leukemia, even if speculative, would be appropriate. Dr. Boorman said there were in-house studies investigating this phenomenon and if something could be added, he would.

Dr. Ryan, the second principal reviewer, said that she had difficulty interpreting the comparisons between the corn oil alone and the dichloromethane groups such that the report should be modified to make clearer the fact that addition of corn oil seems to change the shape of the dose-response curve for effects of dichloromethane. Dr. J. Haseman, NIEHS, said a summary table would be added which would help focus attention on the important pair-wise comparisons and would include a test for interaction. Dr. Ryan commented that conclusions regarding lesions of the mammary tissues were unclear, and given the controvery over diet and breast cancer, this issue may be worth further discussion. Dr. Boorman stated that in female rats receiving dichoromethane in corn oil , there were statistically significant increases in mammary fibroadenomas when comparing the high dose of corn oil with the low dose. However, there was no statistical significance when comparing animals receiving dichloromethane with appropriate corn oil controls.

There ensued a discussion led by Dr. Ward and Dr. Davis as to whether levels of evidence for carcinogenic activity should have been assigned as they were in a typical toxicology and carcinogenesis study. Dr. Boorman commented that this was not designed as a traditional carcinogenicity study in that it was done in a single sex, single species and with a different purpose. Dr. van Zwieten suggested that there might be data from corn oil controls in previous studies for female rats and mice.

Dr. van Zwieten moved that the Technical Report on corn oil, safflower oil, and tricaprylin be accepted with the revisions discussed and with the conclusions as stated in the summary. Dr. Ryan seconded the motion. Dr. Ward offered an amendment that levels of evidence of carcinogenic activity be assigned for the three oils. He proposed **some evidence of carcinogenic activity** for corn oil based on increased incidences of pancreatic acinar cell adenomas, **some evidence of carcinogenic activity** for safflower oil based on increased incidences of pancreatic acinar cell adednomas, and **some evidence of carcinogenic activity** for tricaprylin based on increased incidences of pancreatic acinar cell adenomas and papillomas of the forestomach. Dr. Davis seconded the amendment. In discussion, Dr. William Allaben, NCTR, questioned applying the standard NTP categories to a study that was specifically designed as a research project. Dr. B. Schwetz, NIEHS, cautioned that the study would have been designed differently if the aim was to assess carcinogenicity per se of the oils. For example, controls to match caloric intake would have been included. Dr. Davis said lack of appropriate controls would be a design flaw and would change his viewpoint on assigning levels of evidence. Dr. R. Griesemer, NIEHS, said there would be a summary of this discussion in the Report. Dr. Ward's amendment was defeated by one yes (Ward) to nine no votes. Dr. Davidson offered an amendment that the last part of the second sentence in the Summary which reads "caused proliferative lesions of the exocrine pancreas..." be changed to "caused hyperplasias and adenomas of the exocrine pancreas...". Dr. Boorman suggested that a statement be added noting the forestomach papillomas with tricaprylin, as follows: "Tricaprylin also caused an increase in squamous cell papillomas of the forestomach." Dr. Davidson agreed to the addition. Dr. Carlson seconded the amendment, which was accepted unanimously with ten votes. Dr. Bailey said that based on the study design, the data were inadequate to judge carcinogenicity, and asked that his comment be included in the discussion of the review. Dr. Klaassen asked that an introductory sentence be added to the Summary that would specifically state the rationale for the study. The original motion by Dr. van Zwieten as amended by Dr. Davidson was then accepted unanimously with ten votes.

Oxazepam. Dr. J.R. Bucher, NIEHS, introduced the toxicology and carcinogenesis studies of oxazepam by discussing the uses and rationale for study, describing the experimental design in Swiss-Webster and B6C3F$_1$ mice, reporting on survival and body weight effects, and commenting on compound-related neoplastic and non-neoplastic lesions in both mouse strains. Dr. Bucher reported that in view of the marked enhancement of liver neoplasia in both strains, a number of supplementary studies were performed at NIEHS including a study to evaluate rates of replicative DNA synthesis in the liver, metabolic fate and toxicokinetic studies, and analysis of the frequency of occurrence of an activated H-*ras* oncogene in hepatocellular neoplasms in B6C3F$_1$ mice. The conclusions were that:

> Under the conditions of these 2-year feed studies, there was **clear evidence of carcinogenic activity** of oxazepam in male and female Swiss-Webster mice based on increased incidences of hepatocellular adenomas and carcinomas. There was **clear evidence of carcinogenic activity** of oxazepam in male and female B6C3F$_1$ mice based on increased incidences of hepatoblastoma and hepatocellular adenomas and carcinomas. Increased incidences of hyperplasia of thyroid gland follicular cells in male and female B6C3F$_1$ mice and of follicular cell adenomas in female B6C3F$_1$ mice were also related to oxazepam exposure.
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> Administration of oxazepam to Swiss-Webster mice resulted in centrilobular hepatocellular hypertrophy and increased incidences and severity of systemic amyloidosis. Administration of oxazepam to B6C3F$_1$ mice also resulted in centrilobular hepatocellular hypertrophy.

Dr. Ward, a principal reviewer, agreed with the conclusions. He said it should be noted that in the B6C3F$_1$ mouse study the top two doses exceeded MTD (Maximum Tolerated Dose) guidelines, but despite the severe depression in body weight gain, liver tumors were associated with early mortality and increased food consumption. Dr. Bucher thought this was a reasonable point for further discussion by the Subcommittee. Dr. Ward said it was important to establish in B6C3F$_1$ mice whether the thyroid follicular cell hyperplasia was goiter (diffuse) or focal (not diffuse). Dr. Bucher responded that in the top two doses the hyperplasia was of a diffuse goiter-type. Dr. Ward asked that the appendices associated with the supplementary studies be brought up into the Results section.

Dr. Taylor, the second principal reviewer, agreed with the conclusions. He complimented the inclusion of the mechanistic studies and also urged that the appendices be brought up into the report. Dr. Taylor thought the detailed discussion of chlordiazepoxide genotoxicity was not necessary since little of this agent is metabolized to oxazepam while genotoxicity information might be useful on temazepam which is metabolically converted largely to oxazepam. Dr. Bucher agreed to add genotoxicity information on temazepam if available.

Dr. Ryan, the third principal reviewer, agreed with the conclusions. She said the different patterns of weight gain between male and female Swiss-Webster mice were of some concern and wondered if these patterns were explainable through the varying incidences of toxicity and neoplasia. Dr. Bucher said there was not a clearcut cause and effect relationship that would explain the differences. Dr. Ryan asked why there were no studies done to assess reproductive toxicity since one of

the rationales for this study was use of the drug by pregnant women. Dr. Bucher commented that adequate reproductive and developmental toxicology studies had been done as part of the FDA drug approval process. Dr. Ryan noted that since the 125 ppm dose level in B6C3F$_1$ mice was included in an attempt to produce a blood level in the therapeutic range for humans, interpretation of the findings for humans should be addressed more specifically in the Conclusions. Dr. Bucher said he would try to add a phrase that there were indications in the study that suggested that the amount of oxazepam was sufficient at that dose to influence expression of the neoplastic process.

Dr. Davidson asked that some of the nonneoplastic lesions, notably heart lesions (amyloidosis) in Swiss-Webster mice and testicular lesions in B6C3F$_1$ mice, be summarized in the text along with appropriate statistical analyses. Dr. Bucher explained that since the amyloidosis was a systemic effect such a focus would be putting too much emphasis on the heart lesions. With regard to the testicular lesions, he said it was likely that this was a treatment-related effect but could also be secondary to debilitation of the animal. There was no evidence from the 90-day studies that the testis was a target organ. Dr. Davis thought there needed to be clear presentation in the text of the toxicokinetic studies including area under the curve (AUC) information noting that the extensive amyloidosis in one strain of mice could affect chemical disposition depending on the organs involved. Dr. Bucher said AUC data was included, and noted that young Swiss-Webster mice were used for the toxicokinetic studies so amyloidosis would not have been present.

In comments from the public, Dr. Michael McClain, Hoffman-LaRoche, stated that the existence of thyroid follicular cell hypertrophy along with hyperplasia of a diffuse type provided fairly clear evidence that the thyroid gland effects were probably secondary to hormone imbalance. Dr. Klaassen asked whether serum TSH levels had been measured. Dr. Bucher replied that thyroid hormone status was not determined in the studies done to date, but there were plans to measure TSH and other thyroid hormones in further studies. In response to a question by Dr. Klaassen about measurement of P450 isoforms, Dr. Julian Leakey, NCTR, reported that his laboratory was going to be doing studies in rats and mice treated with oxazepam looking at induction of specific isoforms of P450. Dr. Joseph Contrera, Center for Drug Evaluation and Research, FDA, praised the interaction between FDA and the NTP in the design and conduct of the oxazepam studies.

Dr. Ward moved that the Technical Report on oxazepam be accepted with the revisions discussed and with the conclusions as written for male and female Swiss-Webster mice and male and female B6C3F$_1$ mice, **clear evidence of carcinogenic activity**. Dr. Taylor seconded the motion, which was accepted by nine yes votes with one abstention (van Zwieten) for reasons of company affiliation.

Promethazine Hydrochloride. Dr. K.M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of promethazine hydrochloride by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related nonneoplastic lesions in male rats and decreased incidences of neoplastic lesions in male and female rats and female mice. The conclusions were that:

> Under the conditions of these 2-year gavage studies, there was **no evidence of carcinogenic activity** of promethazine hydrochloride in male or female F344 rats receiving 8.3, 16.6, or 33.3 mg/kg. There was **no evidence of carcinogenic activity** of promethazine hydrochloride in male $B6C3F_1$ mice receiving 11.25, 22.5, or 45 mg/kg. There was **no evidence of carcinogenic activity** of promethazine hydrochloride in female $B6C3F_1$ mice receiving 3.75, 7.5, or 15 mg/kg.
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> The decrease in the incidences of adrenal medulla pheochromocytoma and pituitary gland adenoma in male rats and uterine stromal polyp in female rats were considered to be related to promethazine administration.
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> Promethazine hydrochloride also caused an increased incidence of fatty change in the liver of male rats.

Dr. Bailey, a principal reviewer, agreed with the conclusions. He commented that although mice might have tolerated higher doses, the doses selected were properly based on the results of the 13-week study and were adequate to evaluate carcinogenic potential. He wondered if foreign plant material observed at the sites of nasal inflammatory lesions in 16-day and 13-week studies might have resulted from a change in animal bedding material. Dr. G.N. Rao, NIEHS, said there was a change in brands but the hardwood composition of the bedding did not change.

Dr. Ward, the second principal reviewer, agreed in principle with the conclusions. He also thought that mice might have tolerated higher doses, especially females, and questioned whether reduced incidences of neoplasms reported were associated with chemical exposure but rather could be attributed to reduced survival since most reductions were only in high dose groups and were of marginal statistical significance. Dr. J. Haseman, NIEHS, commented that the causes of some of these negative trends were problematic in that there were also survival and body weight differences. Dr. J. Hailey, NIEHS, opined that the known dopaminergic effects of promethazine could be supportive of an association with chemical exposure.

Dr. Davidson, the third principal reviewer, agreed in principle with the conclusions. She said the evidence for an association between chemical administration and a decreased incidence of adrenal neoplasms in male rats was greater than that for pituitary gland neoplasms in male rats and uterine stromal polyps in female rats, and the final conclusions should reflect these differences.

Dr. Bailey moved that the Technical Report on promethazine hydrochloride be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, **no evidence of carcinogenic activity**. Dr. Carlson seconded the motion. Dr. Davidson offered an amendment to revise the second paragraph of the conclusions to reflect her concerns. The revised paragraph would read as follows: "The decrease in the incidence of adrenal medulla

pheochromocytoma was considered to be related to promethazine hydrochloride administration. The decrease in incidence of pituitary gland adenoma in male rats and uterine stromal polyps in female rats may be related to promethazine hydrochloride administration." Mr. Beliczky seconded the amendment, which was approved by seven yes votes to two no votes (Bailey, Ward) with one abstention (van Zwieten) for reasons of company affiliation. Dr. Ward offered an amendment that a sentence be added to the conclusions stating that mice may have tolerated higher doses. The amendment was tabled for lack of a second. The original motion by Dr. Bailey as amended by Dr. Davidson was then accepted by nine yes votes with one abstention (van Zwieten) for reasons of company affiliation.

## SHORT-TERM TOXICITY STUDIES

Introduction to Studies on the Toxicity of Mixtures.
Dr. Schwetz presented background information on the NTP involvement in mixture studies as preface to the first two mixture toxicity studies to be reviewed by the Subcommittee. He reported that the first discussions at the NIEHS started about 10 years ago and centered on considering what could be done, what should be done, what was scientifically justifiable to do, and what could be done that would be a scientific contribution. To provide guidance, the National Academy of Sciences was asked to form a committee to review approaches for studying the toxicity of mixtures, evaluate human and animal data, and make recommendations. Additionally, an external review committee was formed by the NIEHS and asked to evaluate proposals generated inhouse. This effort led to the decision to study a groundwater mixture and subsequently, pesticide-fertilizer mixtures with the intent being to characterize the toxicity of these mixtures and then evaluate whether this would be the basis for further work with these or other mixtures. Dr. Schwetz introduced Dr. Raymond Yang, Colorado State University, whom he credited with spearheading this effort while at the NIEHS.

Dr. Yang said he would give an overview specifically to address two issues - why we were doing this study and how the mixture was picked. He noted that this was really a group effort and credit should go to a goodly number of people both inside and outside of NTP and NIEHS. This is a special initiative on chemical mixture toxicology carried out through an interagency agreement between the NIEHS/NTP and the Agency for Toxic Substances and Disease Registry (ATSDR) with two parts to the initiative, one being to characterize the toxicity of a mixture of 25 groundwater contaminants frequently detected in and around hazardous waste disposal sites, while the other was to characterize the toxicity of two pesticide fertilizer mixtures. To give perspective to the magnitude of the problem of studying mixtures, he reported that there are currently around 30,000 hazardous waste disposal sites in the country. The problem was to deal with chemically defined mixtures, related to groundwater contamination, with chemicals at environmentally realistic concentrations, and with a potential for lifetime exposure. Dr. Yang concluded by discussing the rationale for the 25 chemicals selected and the dose levels chosen.

A Chemical Mixture of 25 Groundwater Contaminants. Dr. J.R. Bucher, NIEHS, introduced the short-term toxicity studies of a chemical mixture of 25 groundwater contaminants. The report focuses primarily on 26-week drinking water toxicity studies with male and female F344/N rats and B6C3F1 mice with endpoints evaluated including histopathology, clinical pathology, neurobehavioral studies, and reproductive toxicity. Rats receiving levels of potential environmental relevance developed inflammatory lesions in the liver, spleen, lymph nodes, and adrenal gland, as well as evidence of an iron deficiency anemia. The inflammatory lesions could not have been predicted based on the known hepatotoxic effects of the individual components of the mixture. Mice exposed to similar concentrations did not show adverse effects in a standard toxicity study but developed deficits in bone marrow function, evidence of genetic damage, hepatic inflammation and nephropathy, and immunosuppression in other studies which generally included exposures to higher concentrations or exposures of longer duration. A no-observed-adverse-effect level (NOAEL) for histologic injury was 11 ppm in rats; however, no clear evidence for histologic injury was seen in mice exposed to concentrations of the chemical mixture as high as 378 ppm in a standard 26-week study.

Dr. Carlson, a principal reviewer, said his main concern had more to do with the tenor of the report rather than the scientific conclusions for the most part. He thought phrases such as "at environmentally realistic doses" and statements that exposure levels are based on probable human exposures to be misleading as most people and homes are exposed to much lower levels of these chemicals, while phrases such as "most are several orders of magnitude higher than those detected in drinking water sources" were accurate. Dr. Bucher said he would look carefully at the entire report and try to make clear when we are talking about environmentally realistic doses and when we mean finished drinking water and groundwater sources. Also, a table would be put in the abstract defining the composition of the mixture. Dr. Carlson did not agree with the basis for determining the NOAEL in rats. Dr. Bucher explained that there were two types of inflammatory lesions seen in the liver of rats, one typically seen in controls and a second, more severe lesion, characteristic of the dosed animals.

Dr. Davis, a second principal reviewer, complimented the Program's two pronged approach to determining the toxicity of chemical mixtures, i.e., first conducting studies of well-defined mixtures, and secondly evaluating mechanisms responsible for effects other than additivity. He had concerns about the exclusion of cyanide, 2,4-dichlorophenoxyacetic acid (2,4-D), and vinyl chloride from the final mixture. Dr. Bucher said a better explanation would be added for the exclusion of the three compounds that were not stable in this mixture.

Dr. van Zwieten wondered if a summary table could be added that would indicate the known toxicity of the individual components so the reader could compare the toxicity of the mixture against individual chemical toxicities. Dr. Schwetz said that had been considered but noted that for many chemicals the type of toxicity is different at different dose levels and so as not to mislead readers into thinking that we tested a particular chemical at a level which would be toxic , we decided not to include such a table. Dr. Yang commented that with the exception of the liver lesions, the other toxic effects are subtle.

2-Chloronitrobenzene and 4-Chloronitrobenzene. Dr. J.R. Bucher, NIEHS, introduced the short-term toxicity studies of 2-chloronitrobenzene and 4-chloronitrobenzene by reviewing the uses, experimental design, and results. Toxicity studies were performed by exposing male and female F344/N rats and $B6C3F_1$ mice to the chemicals by whole body inhalation for two and 13-weeks. In separate studies, the dermal absorption of the chemicals was compared, and the absorption, distribution, metabolism, and excretion were partially characterized following oral administration to male rats.

Inhalation exposure of rats and mice to 2- or 4-chloronitrobenzene resulted in methemoglobin formation and oxidative damage to red blood cells, leading to a regenerative anemia (rats only) and a recognized spectrum of tissue damage and changes secondary to erythrocyte injury. In addition, numerous other lesions occurred following exposure to both chemicals that were considered primary toxic effects. These included renal hyaline droplet accumulation and testicular atrophy in male rats exposed to 4-chloronitrobenzene and hyperplasia of the repiratory epithelium in rats exposed to 2-chloronitrobenzene. A NOAEL for rats was not determined, since increases in methemoglobin and histopathologic changes occurred at exposure concentrations as low as 1.1 ppm for 2-chloro and 1.5 ppm for 4-chloronitrobenzene in 13-week studies. An NOAEL for histopathologic injury in mice was 4.5 ppm for 2-chloronitrobenzene and 6 ppm for 4-chloronitrobenzene. Significant percutaneous absorption of both isomers was demonstrated in rats. Oral absorption was somewhat higher than dermal with both chemicals, and metabolism was complicated with over 20 unidentified metabolites isolated from the urine of rats given either 2- or 4-chloronitrobenzene.

Dr. van Zwieten, a principal reviewer, thought the report was well written and the results clearly presented. He encouraged inclusion in the report of photomicrographs of representative lesions, since a number of relatively uncommon changes in a variety of tissues were described. Dr. Bucher said we would try to make a selection of some appropriate photomicrographs to include.

Mr. Beliczky, a second principal reviewer, noted that since both of the chloronitrobenzenes are considered highly toxic materials, laboratory health and safety concerns should be referenced, and wondered how the chamber effluent was handled. Dr. Bucher said the laboratories follow NTP health and safety guidelines. The effluents from the chambers are passed through the appropriate type of filtration systems and the stacks are monitored to make sure that emissions don't exceed Federal guidelines. More information on the particulars for this study would be put in the report. Mr. Beliczky stated that because of the large production volumes of the chloronitrobenzenes and the potential health risk due to both airborne and percutaneous exposures, these chemicals should proceed to full carcinogenesis bioassays. Dr. Bucher said that 4-chloronitrobenzene was being considered for further studies.

Cupric Sulfate. Dr. Charles Hébert, NIEHS, introduced the short-term toxicity studies of cupric sulfate by reviewing the use and rationale for study, experimental design, and results. Toxicity studies were conducted in male and female F344/N rats and $B6C3F_1$ mice by the drinking water (2-week studies only) and dosed feed routes (2-week and 13-week studies). Animals were evaluated for hematology, clinical chemistry, urinalysis, reproductive toxicity, tissue metal accumulation, and histopathology.

Exposure of rats to cupric sulfate in feed or drinking water resulted in significant hepatic and renal damage. The primary histologic lesion in rats was an increase in the size and number of proteinaceous droplets in the epithelial cytoplasm and lumen of the kidney proximal convoluted tubule. Hyperplasia with hyperkeratosis of the forestomach epithelium was seen in both species. Hematologic and clinical chemistry alterations noted in rats in the 13-week study, along with histologic changes in bone marrow noted in the 2-week feed study, are indicative of a microcytic anemia with a compensatory bone marrow response. Mice appear to be much more resistant to the toxic effects of cupric sulfate than rats.

Dr. Davis, a principal reviewer, asked that if atrophy and cellular depletion in many tissues of mice occurred due to dehydration and dehydration was due to cupric sulfate then should not atrophy and cellular depletion be due to cupric sulfate. Dr. Hébert opined that they were treatment-related effects not specifically related to cupric sulfate because atrophy and cellular depletion were the sort of effects that would be expected from any compound that would cause dehydration. Dr. Davis inquired as to how the finding of increased aspartate aminotransferase, a serum enzyme, was associated with renal tubule epithelial damage. Dr. Hébert responded that the enzyme was measured in the urine where it is used as a measure of damage to the renal tubular epithelium.

Dr. Bailey, a second principal reviewer, considered this to be a thorough and well prepared report which presents a large body of information in a well organized and succinct manner. He commented on the increased copper levels and reduced calcium levels in the testis of male rats in the 13-week studies and asked whether these kinds of changes could have influenced reproductive function. Dr. Hébert replied that just on the basis of membrane damage and cellular necrosis from these changes one would expect to see some sort of reproductive effects but there were no alterations in any of the reproductive parameters measured.

Ethylene Glycol Ethers. Dr. M.P. Dieter, NIEHS, introduced the short-term toxicity studies of three ethylene glycol ethers -- 2-methoxyethanol, 2-ethoxyethanol, and 2-butoxyethanol -- by reviewing the use and rationale for study, experimental design, and results. General toxicity and supplemental clinical pathology and reproductive toxicity studies were conducted in male and female F344/N rats and $B6C3F_1$ mice exposed to each of the glycol alkyl ethers in the drinking water for either two or 13-weeks. Stop-exposure protocols were used to evaluate persistence of spermatotoxicity. Rank order of toxicity for the glycol alkyl ethers was 2-methoxyethanol > 2-ethoxyethanol > 2-butoxyethanol. Based on survival, decreased body weight gains, and histopathological effects for each glycol ether tested, the toxicity was greater in rats than mice. The major target organs for toxicity were the testes in male rats and mice and the hematopoietic system in rats and mice of each sex.

Dr. Bailey, a principal reviewer, said this was a well written and well organized report. He thought it would be useful to include the various exposure limits for these chemicals in the Introduction and not just the references to the NIOSH criteria documents. Dr. Bailey inquired as to what type of cages were used in the studies. He said this was relevant since some of the glycol ethers acid metabolites excreted via urine could have similar toxicological properties as the parent compound and result in additional exposure. The cages had a wire mesh floor with the bedding beneath which is standard caging procedure for NTP studies. Dr. Dieter agreed to do this.

Dr. Carlson, a second principal reviewer, considered this to be a clear and well written report, especially considering it was comparing three different compounds at two time periods in two species. He requested that data be given in the report for the thymus and testis weights in the 2-week studies since these changes were considered very important in the discussion section. Dr. Dieter said a table with this data would be included in the final report. Dr. Carlson said there was an inconsistency in rank ordering the toxicity of the three glycol ethers as stated while indicating that the no-observed-adverse-effect levels (NOAELs) were not in that order. Dr. Dieter said this apparent inconsistency had been reviewed and that the rank order in mice for both held true. For rats, there was some equivocation between the NOAELs for ethoxyethanol and butoxyethanol depending on the criteria used for the NOAEL but from an overall standpoint considering the various end points of toxicity there was consistency.

There were two speakers from the audience representing the Chemical Manufacturers Association Glycol Ethers Panel, Toxicology Research Task Group. Dr. Ralph Gingell, Shell Oil Company, requested that the data on the methyl and ethyl ethers be separated into a different report as they are clearly more toxic especially with respect to testicular effects and developmental toxicity than the butyl ether, and further, their use is decreasing while that for the butyl ether is increasing especially in consumer products. He noted that the hematotoxicity reported for the butyl ether has not been reported in humans. Dr. Rodney Boatman, Eastman Kodak Company, questioned the speculation in the report that the minor acidic metabolites of the ethers may be contributing to their toxicity. He was not aware of any studies demonstrating such toxicity, and if there are reports they should be referenced.

Pesticide/Fertilizer Mixtures. Dr. J. R. Bucher, NIEHS, introduced the short-term toxicity studies of pesticide/fertilizer mixtures noting that the mixtures were representative of groundwater contamination found in California and Iowa. The California mixture was composed of aldicarb, atrazine, 1,2-dibromo-3-chloropropane, 1,2-dichloropropane, ethylene dibromide, simazine, and ammonium nitrate. The Iowa mixture contained alachlor, atrazine, cyanazine, metolachlor, metribuzin, and ammonium nitrate. The mixtures were administered in the drinking water for 26 weeks to F344/N rats and B6C3F$_1$ mice at concentrations ranging from 0.1X to 100X where 1X represented the median concentrations of the individual chemicals found in studies of groundwater contamination from normal agricultural activities.

All rats survived to the end of the studies, there were no significant effects on body weight gain, and there were no clear clinical signs of toxicity or neurobehavioral effects. There were no clear adverse effects as shown by clinical pathology or of the reproductive system. In studies with mice, one male receiving the California mixture at the highest dose died early while one control female and one female receiving the Iowa mixture at the highest dose also died early. An association with consumption of the mixtures could not be determined. Water consumption and body weight gains were not affected, nor were there signs of toxicity noted in clinical observations, neurobehavioral assessments, reproductive or developmental toxicity assessments.

Dr. Davidson, a principal reviewer, wondered as to the overall relevance of these studies since it would not be possible to extrapolate this information to other combinations of contaminants. She asked for an explanation as to why 26-weeks was the exposure period instead of the more standard 13-weeks. Dr. Bucher said the mixtures were administered for 26 weeks simply to increase the sensitivity of the assay for detecting adverse effects. Dr. Davidson requested that a table be included in the Introduction listing CAS numbers, chemical formula, molecular weight, physical state, and solubility of the components of each mixture. Dr. Bucher agreed to add such a table.

Dr. Taylor, a second principal reviewer, thought this was a straightforward study supporting the absence of adverse effects of these mixtures. He asked for inclusion of information as to how the concentrations were selected. Dr. Bucher said more information would be included. Dr. Ward commented that biochemical markers should have been included as they might be more sensitive for detecting subtle toxicity.

Dr. R. Griesemer, NIEHS, commented that the NTP and the NIEHS are asked frequently by Congress and others to explore the potential toxicity of environmental exposures which are usually either mixtures or mixed exposures. We are asked to look at complex situations and complex exposures with often little scientific basis for knowing how to proceed. Dr. Yang said the results of the two studies should be reassuring to the public.