Board of Scientific Counselors
National Toxicology Program

Summary Minutes
from

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

on

July 9-10, 1991

Research Triangle Park, North Carolina

The review meeting began at 9:00 a.m. on July 9 and 10 in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee are: Drs. Daniel Longnecker (Chairperson), Paul Bailey, Gary Carlson, Harold Davis, Robert Garman, Jay Goodman, David Hayden, Curtis Klaassen, Barbara McKnight, Ellen Silbergeld, Lauren Zeise, and Mr. Louis Beliczky. Dr. Silbergeld was unable to attend the meeting. These minutes have been reviewed and approved by all members of the Subcommittee present. 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; FTS: 629-3419. Subsequently, they may be purchased from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, Virginia 22161, (703) 487-4650.

The next NTP technical reports peer review meeting will be held November 21, 1991, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, (919) 541-3971; FTS 629-3971.

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TECHNICAL REPORTS REVIEW SUBCOMMITTEE MEETING

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gamma-Butyrolactone. Dr. S.L. Eustis, NIEHS, introduced the toxicology and carcinogenesis studies of gamma-butyrolactone by discussing the uses, describing the experimental design, reporting on survival and body weight effects, and commenting on non-neoplastic lesions in mice. The conclusions were that:

> Under the conditions of these 2-year studies, there was no evidence of carcinogenic activity of gamma-butyrolactone in male F344/N rats given 112 or 225 mg/kg or in female F344/N rats given 225 or 450 mg/kg in corn oil by gavage. There was equivocal evidence of carcinogenic activity of gamma-butyrolactone in male B6C3F1 mice given 262 or 525 mg/kg in corn oil by gavage based on increased incidences of adrenal medullary pheochromocytomas and hyperplasia in the low dose group. The sensitivity of the study in male mice to detect a carcinogenic effect was reduced by the low survival of the high dose group. There was no evidence of carcinogenic activity of gamma-butyrolactone in female B6C3F1 mice given 262 or 525 mg/kg in corn oil by gavage.
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> A decreased incidence of hepatocellular neoplasms in dosed male mice, and decreased incidences of mammary gland fibroadenomas and cysts and pituitary cysts in female rats were associated with the administration of gamma-butyrolactone.

Dr. Carlson, a principal reviewer, agreed with the conclusions. However, he said he could be convinced that the study in male mice was inadequate due to poor survival. He said the conclusions should note that the incidence of mononuclear cell leukemia in male rats occurred with a significant negative trend. Dr. Eustis agreed to add a statement about the leukemias.

Dr. Goodman, the second principal reviewer, agreed in principle with the conclusions. Since the level of evidence in male mice was based on an increased incidence of adrenal tumors only in the low dose group, he thought the sentence in the conclusions should be changed to read: "There was equivocal evidence of carcinogenic activity of gamma-butyrolactone in male B6C3F1 mice in the low dose group (262 mg/kg), but not in the high dose group (525 mg/kg), based upon a non-statistically significant increase in the incidence of adrenal medullary pheochromocytomas and hyperplasia." Dr. Eustis said the definition of equivocal evidence presumes a lack of statistical significance. Dr. Goodman asked that a rationale be given for using the Drosophila protocol involving the sex-linked recessive lethal test.

Dr. Hayden, the third principal reviewer, agreed in principle with the conclusions. He noted the low survival rate for high dose male mice, and the resultant lower sensitivity for detecting a carcinogenic effect. He wondered at what point low numbers of surviving animals rendered a study group inadequate,

versus only less sensitive for evaluating carcinogenic potential. Dr. Eustis said there are no hard and fast rules about deciding when survival is adequate, and this was certainly a borderline case. Dr. R. Griesemer, NIEHS, said this could be viewed as a one-dose study since survival was certainly adequate in the low-dose male mice group.

Dr. Carlson moved that the Technical Report on gamma-butyrolactone be accepted with the revisions discussed and with the conclusions as written in the Report for male and female rats and female mice, no evidence of carcinogenic activity, and for male mice, equivocal evidence of carcinogenic activity. Dr. Goodman seconded the motion, which was accepted unanimously with ten votes.

C.I. Pigment Red 3. Dr. R.D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of C.I. Pigment Red 3 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 and nonneoplastic lesions in rats and mice. The conclusions were that:

> Under the conditions of these 2-year feed studies, there was some evidence of carcinogenic activity of C.I. Pigment Red 3 in male F344/N rats as exhibited by increased incidences of benign pheochromocytomas of the adrenal gland. The marginal increase in the incidence of squamous cell papillomas of the skin and Zymbal's gland carcinomas may have been related to C.I. Pigment Red 3 administration. There was some evidence of carcinogenic activity of C.I. Pigment Red 3 in female F344/N rats as indicated by the increased incidence of hepatocellular adenomas. There was some evidence of carcinogenic activity of C.I.Pigment Red 3 in male B6C3F1 mice as exhibited by the increased incidences of tubule adenomas of the renal cortex and follicular cell adenomas of the thyroid gland. There was no evidence of carcinogenic activity for C.I. Pigment Red 3 in female B6C3F1 mice that received 12,500, 25,000, or 50,000 ppm.
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> Incidences of mononuclear cell leukemia and preputial gland tumors in male rats and mononuclear cell leukemia, mammary gland fibroadenoma, and clitoral gland tumors in female rats occurred with negative trends. The incidences of liver foci were markedly increased in male and female rats. The severity of chronic nephropathy was increased in male rats and to a lesser extent in female rats. An increase in the severity of nephropathy as well as cytomegaly (karyomegaly) of renal tubule epithelium was observed in male mice. Thyroid follicular cell hyperplasia occurred with an increased incidence in male and female mice receiving C.I. Pigment Red 3.

Dr. Davis, a principal reviewer, agreed with the conclusions. He asked for clarification as to why 50,000 ppm was the upper limit in a feed study, and why, in view of the chemical's uses, the dermal route of exposure was not chosen. Dr. Irwin said there was a longstanding NTP policy that dietary levels exceeding 5% (50,000 ppm) would compromise the nutritional status of the animal. Dr. Davis commented on the inclusion of statements that there were no clinical findings indicative of toxicity, and asked how this could be reconciled with reductions in body weight gain of more than 10%. Dr. Irwin agreed that reduced body weight gain could be an indicator of toxicity but, perhaps as a matter of semantics, was not classified as a clinical finding.

Dr. Klaassen, the second principal reviewer, agreed with the conclusions.

Dr. Bailey, the third principal reviewer, agreed with the conclusions. He also questioned whether dermal exposure might have been appropriate, and thought percutaneous absorption data might have been useful. Dr. Irwin explained that pigments have to be dissolved in an organic solvent for dermal administration and often a residue of the pigment remains on the skin. A variable amount of the residual chemical will be ingested by the animal through natural grooming. Thus, the oral study is likely to be more quantitative and should provide a better measure of carcinogenic potential.

Dr. McKnight suggested that for Zymbal's gland tumors in male rats, a statistically significant trend test and an incidence in the high-dose group which exceeded that in any historical control group supported these tumors being considered some evidence. These tumors were in the conclusion as "may have been related to chemical administration."

Dr. Davis moved that the Technical Report on C.I. Pigment Red 3 be accepted with the revisions discussed and with the conclusions as written for male and female rats and male mice, some evidence of carcinogenic activity, and for female mice, no evidence of carcinogenic activity. Dr. Goodman seconded the motion. Dr. McKnight offered an amendment that Zymbal's gland carcinomas be included as supporting some evidence of carcinogenic activity in male rats. Dr. Zeise seconded the amendment which was defeated by two yes (McKnight, Zeise) to eight no votes. The original motion by Dr. Davis was then accepted unanimously with ten votes.

4,4'-Diamino-2,2'-Stilbenedisulfonic Acid. Dr. J.R. Hailey, NIEHS, introduced the toxicology and carcinogenesis studies of 4,4'-diamino-2,2'-stilbenedisulfonic acid (amsonic acid) 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 rats.The conclusions were that:

> Under the conditions of these 2-year feed studies, there was no evidence of carcinogenic activity of 4,4'-diamino-2,2'-stilbenedisulfonic acid, disodium salt, in male or female F344/N rats receiving 12,500 or 25,000 ppm. There was no evidence of carcinogenic activity in male or female B6C3F1 mice receiving 6,250 or 12,500 ppm 4,4'-diamino-2,2'-stilbene-disulfonic acid, disodium salt.

Dr. Hayden, a principal reviewer, agreed with the conclusions. To emphasize the lack of toxicity, especially in mice, he thought a statement might be added to the conclusion indicating there was no evidence of toxic or nonneoplastic activity in male or female mice. Dr. Hailey said such a statement would be added to the Abstract.

Dr. Zeise, the second principal reviewer, agreed in principle with the conclusions. She said that it should be noted that male and female rats may have been able to tolerate higher doses. Dr. Hailey said he agreed that females could have tolerated higher doses but considered the doses in males to be adequate. Dr. Zeise commented that the summary tables provided combined incidence data for mammary tumors (adenomas, fibroadenomas, and adenocarcinomas) indicating significantly increased levels for female rats, and that this finding should be addressed in the report. Dr. Hailey said they would eliminate the combination since the morphological continuum seen with many neoplastic processes is not seen with fibroadenomas.

Mr. Beliczky, the third principal reviewer, agreed with the conclusions. He thought it would be of value for NIOSH to evaluate the facility which manufactured the amsonic acid in view of reported sexual dysfunction in workers and animal studies that exhibited uterotropic effects.

Dr. Hayden moved that the Technical Report on 4,4'-diamino-2,2'-stilbenedisulfonic acid be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, no evidence of carcinogenic activity. Mr. Beliczky seconded the motion, which was accepted unanimously with ten votes.

Ethylene Glycol. Dr. M.R. McDonald, NIEHS, introduced the toxicology and carcinogenesis studies of ethylene glycol by discussing uses of the chemical and rationale for study, describing the experimental design, reporting on survival and body weight effects, and reviewing nonneoplastic lesions in mice. The conclusions were that:

> Under the conditions of these 2-year feed studies, there was no evidence of carcinogenic activity for ethylene glycol in male B6C3F1 mice receiving 6,250, 12,500, or 25,000 ppm, or female B6C3F1 mice receiving 12,500, 25,000, or 50,000 ppm for up to 103 weeks. Administration of ethylene glycol resulted in hepatocellular hyaline degeneration in male mice fed diets containing 12,500 or 25,000 ppm ethylene glycol and female mice fed diets containing 50,000 ppm ethylene glycol. An increased incidence of medial hyperplasia of small pulmonary arteries and arterioles occurred in female mice fed diets containing 12,500, 25,000, or 50,000 ppm ethylene glycol.

Dr. Klaassen, a principal reviewer, agreed with the conclusions. He noted that water was not used as the route of administration because of the concern that ethylene glycol might decrease consumption, and wondered if, the chemical might increase water consumption. He requested that there be a statement included as to why studies were not performed in rats.

Dr. Hayden, the second principal reviewer, agreed with the conclusions. He asked for an explanation as to why gavage was not used. Dr. McDonald said oral dosing other than gavage better mimicked usual human exposure. Dr. Hayden stated that a reference to hepatocellular erythrophagocytosis should be modified in that the evidence for erythrocyte inclusions was inconclusive. Dr. McDonald said this discussion would be modified. Dr. Hayden inquired as to whether there might be a common link between the structure of ethylene glycol and its metabolites and other chemicals that have been associated with the presence of hepatocellular hyaline degeneration. Dr. McDonald responded that the lesions had been observed in studies with at least three other chemicals, doxylamine, pentachloroanisole, and polybrominated biphenyls, none of which were structurally related to ethylene glycol.

Dr. Carlson, the third principal reviewer, agreed with the conclusions. He also wondered why water was not used as the vehicle since due to the chemical's sweet taste he would expect consumption to be increased. Dr. McDonald commented that ethylene glycol is known to cause severe progressive renal disease in other species. The end stage disease accompanied by polyuria would be expected to lead to excess consumption of water and a resultant overdosage of ethylene glycol by this route. Dr. Carlson asked whether there was information on bioavailability of ethylene glycol from the feed. Dr. McDonald said there were no data. She thought the high incidence of chemically associated systemic lesions supported adequate bioavailability.

Mr. Beliczky commented that occupational exposure would more likely be by inhalation or dermal absorption. He noted that epichlorhydrin was among the two percent impurities and this should be referenced. Dr. Janet Haartz, NIOSH, reported that occupational exposure to ethylene glycol is rather extensive, with potential exposure in the workplace of over one-and-a-half million people.

Dr. Klaassen moved that the Technical Report on ethylene glycol be accepted with the revisions discussed and with the conclusions as written for male and female mice, no evidence of carcinogenic activity. Dr. Bailey seconded the motion, which was accepted unanimously with ten votes.

HC Yellow 4. Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of HC Yellow 4 by discussing the uses, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic lesions in male rats and nonneoplastic lesions in mice. The conclusions were that:

> Under the conditions of these 2-year feed studies, there was equivocal evidence of carcinogenic activity of HC Yellow 4 in male rats based on the increased incidence of pituitary gland adenomas. There was no evidence of carcinogenic activity in female rats given 5,000 or 10,000 ppm HC Yellow 4 in feed for 2 years. There was no evidence of carcinogenic activity in male or female mice given 5,000 or 10,000 ppm HC Yellow 4 in feed for 2 years.
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> There was a chemical-related increase in the incidence of thyroid gland pigmentation and follicular cell hyperplasia in mice.

Dr. Zeise, a principal reviewer, agreed in principle with the conclusions. She thought the conclusions should note that male and female rats could have tolerated significantly higher doses. Dr. Zeise said that the increased incidence of uterine stromal polyps in female rats should be considered "may have been related to chemical administration", unless there are better reasons for discounting them than that the incidence in treated animals falls outside the range of overall NTP historical controls. Dr. Dunnick commented that more historical control data would be added and that there were no supporting nonneoplastic effects, providing further evidence that these lesions probably were not chemically related. Dr. Haseman added that the high dose uterine polyp response was similar to the historical control mean from previous studies at this laboratory. Further, based on the results of previous NCI/NTP studies, it would be unusual for a chemical to induce just uterine polyps.

Dr. Carlson, the second principal reviewer, agreed with the conclusions.

Dr. Garman, the third principal reviewer, agreed with the conclusions. Because of the prominent treatment-related increased frequency of thyroid follicular cell hyperplasia in the 2-year studies in mice, he thought it appropriate to add frequency figures to the summary table in the Abstract. Both Dr. Carlson and Dr. Hayden asked that degrees of pigmentation and hyperplasia of the thyroid glands in mice be added to the tables for the 13-week and 2-year studies.

Dr. Zeise moved that the Technical Report on HC Yellow 4 be accepted with the revisions discussed and with the conclusions as written for male rats, equivocal evidence of carcinogenic activity, and for female rats and male and female mice, no evidence of carcinogenic activity, and with addition of a statement that "male and female rats may have been able to tolerate higher doses". Dr. Garman seconded the motion. Dr. Goodman offered an amendment that the added statement be removed. Dr. Klaassen seconded the amendment which was accepted by seven yes to three no votes (Carlson, McKnight, Zeise). Dr. McKnight offered an amendment to add a statement to the conclusions that "male rats may have been able to tolerate a higher dose". Dr. Zeise seconded the amendment which was accepted by seven yes to three no votes (Beliczky, Goodman, Hayden). Dr. Zeise's amended motion was then accepted unanimously with ten votes.

Mercuric Chloride. Dr. G.A. Boorman, NIEHS, introduced the toxicology and carcinogenesis studies of mercuric chloride by discussing the uses and rationale for study, describing the experimental design including analysis of tissue and organ levels of mercury during a 6-months study, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in mice and rats. In summary, he thought the main concern with mercuric chloride should be toxicity, more so than carcinogenicity. The conclusions were that:

> Under the conditions of these 2-year gavage studies, there was some evidence of carcinogenic activity of mercuric chloride in male F344/N rats based on the increased incidence of squamous cell papillomas of the forestomach. An increased incidence of thyroid follicular cell adenomas and carcinomas may have been related to mercuric chloride exposure. There was equivocal evidence of carcinogenic activity of mercuric chloride in female F344/N rats based on two squamous cell papillomas of the forestomach. There was equivocal evidence of carcinogenic activity of mercuric chloride in male B6C3F1 mice based on the occurrences of two renal tubule adenomas and renal tubule adenocarcinoma. There was no evidence of carcinogenic activity in female B6C3F1 mice receiving 5 or 10 mg/kg of mercuric chloride.
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> Nonneoplastic lesions associated with exposure to mercuric chloride included increased severity of nephropathy in male rats and male and female mice. There was an increased incidence of renal tubule hyperplasia in male rats. The incidence of forestomach hyperplasia was increased in dosed male and female rats. There were increased incidences of inflammation of the nasal mucosa in rats and of olfactory epithelial metaplasia in mice that were associated with mercuric chloride exposure.

Mr. Beliczky, a principal reviewer, agreed in principle with the conclusions. He thought the statement regarding thyroid neoplasms in male rats should be deleted. He expressed concern that carcinogenicity data generated from the studies appeared to be compromised by the chemical toxicity, particularly that in the kidney. Mr. Beliczky stated that if the severe toxicity of the chemical clearly limited the sensitivity of the study to detect carcinogenic effects, then the study design was limited or should have been modified after the six-month study. Dr. Boorman agreed that in some cases toxicity may have interfered with the ability to assess carcinogenicity.

Dr. Garman, the second principal reviewer, agreed with the conclusions. In view of the importance of the renal toxicity and the continuum which exists between hyperplasia, adenoma and carcinoma, he suggested inclusion of a photomicrograph of a representive lesion of renal tubular hyperplasia.

Dr. Goodman, the third principal reviewer, agreed with the conclusions with the exception that he thought the second sentence should be qualified with the word 'marginally' to read: "A marginally increased incidence of thyroid follicular cell adenomas and carcinomas may have been related to mercuric chloride exposure." Dr. Boorman said we would consider such a change. Dr. Goodman commented on the statement in the genetic toxicology section that the induction of a high number of complex chromosomal aberrations implicated mercuric chloride as a major cause of damage, as opposed to cytotoxicity, which would be

expected to produce mainly simple breaks. He praised this as the sort of insight he would like to see more often, and asked that a reference or two be added. Dr. Boorman said there were two pertinent references that would be cited.

Dr. Klaassen suggested that there be an expanded discussion of how the three forms of mercury -- mercury vapor, organic mercury, and inorganic mercury salts -- differ in toxicity. Dr. Boorman said a paragraph would be added. Dr. Carlson commented on the poor survival in dosed male rats and wondered about the adequacy of the study in male rats. Dr. Haseman noted that survival to week 90 was about 60% in both dosed groups so a majority of animals survived long enough to be considered at risk for tumors. Further, since there was a positive effect for carcinogenicity, the low survival is less of a concern. Dr. Zeise thought the level of evidence in female rats should have been some evidence based on the two squamous cell papillomas in the high dose group with supporting hyperplasia and similar increases in male rats. Dr. Boorman responded that based on only two tumors the staff did not think there was an unequivocal association with the chemical. Dr. McKnight argued that the three renal tubule neoplasms in high dose male mice supported some evidence, particularly in view of zero incidence in concurrent controls and historical controls for water gavage studies. Dr. Boorman said the staff had considered this level; however, step sections of the kidneys failed to uncover any additional lesions in male or female mice, weakening the support for a chemically associated effect.

Mr. Beliczky moved that the Technical Report on mercuric chloride be accepted with the revisions discussed, with the conclusions as written for male rats, some evidence of carcinogenic activity, for female rats and male mice, equivocal evidence of carcinogenic activity, and for female mice, no evidence of carcinogenic activity, and with deletion of the second sentence: "An increased incidence of thyroid follicular cell adenomas and carcinomas may have been related to mercuric chloride exposure." The motion was tabled for lack of a second. Mr. Beliczky then moved that the conclusions be accepted as written including the second sentence. Dr. Garman seconded the motion. Dr. Goodman offered an amendment that the sentence have "marginally" inserted as its second word. Dr. Klaassen seconded the amendment, which was accepted by nine yes to one no votes (Zeise). Dr. Zeise offered an amendment that the conclusion for male mice be changed to some evidence of carcinogenic activity. Dr. McKnight seconded the amendment, which was defeated by eight no to two yes votes (McKnight, Zeise). Dr. Zeise offered an amendment that the conclusions for female rats be changed to some evidence of carcinogenic activity. The amendment was tabled for lack of a second. Mr. Beliczky's second motion to accept the conclusions as written with the second sentence amended to include "marginally" as the second word was then accepted by nine yes to one no votes (Zeise).

p-Nitrophenol. Dr. C.C. Shackelford, NIEHS, introduced the toxicology and carcinogenesis studies of p-nitrophenol by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on the lack of compound-related neoplastic and nonneoplastic lesions in mice. The studies were terminated at 18 months because of poor survival due to generalized amyloidosis and secondary kidney failure in both dosed and control animals. The conclusions were that:

> Under the conditions of these 18-month dermal studies, there was no evidence of carcinogenic activity in male or female Swiss-Webster mice receiving 40, 80, or 160 mg/kg p-nitrophenol.

Dr. Bailey, a principal reviewer, agreed with the conclusions. He questioned the sensitivity of Swiss-Webster mice with regards to p-nitrophenol toxicity (e.g., oral administration) as compared to other strains of mice, and whether parallels could be drawn between the Swiss-Webster and other strains of mice currently used in classical carcinogenicity testing. Dr. Shackelford said there was no information in the literature on such studies. Dr. Bailey also wondered whether clinical chemistry had been done. Dr. Shackelford explained that because of the large number of animals moribund at 65 weeks, the time when blood samples are taken for clinical chemistry, there were insufficient hematologic data to report.

Dr. Davis, the second principal reviewer, agreed with the conclusions. He questioned the rationale for using Swiss-Webster mice. Dr. R. Irwin, NIEHS, said that the U.S. Army specifically requested that the study be done in this strain. Dr. Davis noted a statement that a higher dose, than 160 mg/kg, could not have been tolerated given results in an earlier unpublished 13-week study at 175 mg/kg and asked for clarification.

Mr. Beliczky, the third principal reviewer, agreed with the conclusions although he considered the Swiss-Webster mouse a poor choice of animal, thus precluding any final judgment regarding either toxicity or carcinogenicity of p-nitrophenol. He said another study using a different strain of animal should be considered. Dr. S. Eustis, NIEHS, responded that for dermal carcinogenicity studies, the usual NTP rodent species, B6C3F1 mouse and Fischer 344 rat, may not be the best strains to use because they are relatively resistant to dermal carcinogenesis. He thought that other studies by another route in these strains would be more useful.

There was a lengthy discussion on the merits of exposure by the feed route vs. dermal application and the point was made that when a chemical is given by one of these routes there is usually some degree of inadvertent exposure by the other route. Dr. R. Griesemer, NIEHS, commented that the design of the dermal study might be quite different depending on whether the concern was with skin as a target site or skin as a portal of entry.

Dr. Bailey moved that the Technical Report on p-nitrophenol be accepted with the revisions discussed and with the conclusions as written for male and female Swiss-Webster mice, no evidence of carcinogenic activity. Dr. Davis seconded the motion, which was accepted unanimously with ten votes.

Polysorbate 80. Dr. M.P. Jokinen, NIEHS, introduced the toxicology and carcinogenesis studies of polysorbate 80 by discussing the uses and rationale for study, describing the chemistry and experimental design, and commenting on compound-related neoplastic and nonneoplastic lesions in mice and rats. The conclusions were that:

> Under the conditions of these 2-year feed studies, there was equivocal evidence of carcinogenic activity of polysorbate 80 in male F344/N rats based on increased incidences of pheochromocytomas of the adrenal medulla. There was no evidence of carcinogenic activity in female F344/N rats or in male or female B6C3F1 mice given 25,000 or 50,000 ppm polysorbate 80 in feed for 2 years.
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> Administration of polysorbate 80 was associated with inflammation and squamous hyperplasia of the forestomach in male and female mice, and with ulcers of the forestomach in female mice.

Dr. Garman, a principal reviewer, agreed with the conclusions. He asked for clarification of the criteria for distinguishing between benign and malignant pheochromocytomas. Dr. Jokinen said the criteria for malignancy is invasion through the capsule. Dr. Garman also asked for clarification as to why the upper end of the historical control range for pheochromocytomas in male rats was cited as 48% in the report, yet the upper end cited by Dr. Jokinen in his remarks was 65%. Dr. J. Haseman, NIEHS, explained that 48% was the upper limit on the current database while 65% was cited from an earlier NTP study to give perspective to the fact that rates had been higher in the past. He pointed out a paragraph in the Discussion where this was explained.

Dr. Bailey, the second principal reviewer, agreed with the conclusions. He commented on the large temperature excursion in the animal room occurring during the 2-year study, noting that it was only during a 2-day period, and asked for a statement in the report as to any possible impact on results of the study. Dr. Jokinen agreed to add a statement as to the limited time of the excursion and the likelihood there was no effect on the study.

Dr. Davis, the third principal reviewer, agreed with the conclusions. He said it was not clear to him how much significance was placed on the occurrence of adrenal hyperplasia in low dose male rats or, if adrenal hyperplasia is part of a continuum toward neoplasia, why there were not increases in the high dose group. Dr. S. Eustis, NIEHS, said that if both a hyperplasia and a neoplasm were present in the same adrenal gland only the neoplasm was diagnosed and reported. This was mainly a matter of practicality since the small size of the adrenal medulla made it difficult to determine whether another slight lesion was part of the pheochromocytoma or a separate lesion.

Dr. Carlson commented on the occurrence of splenic sarcomas in two high-dose male rats, an incidence which exceeded the current historical control range for dosed food studies. Dr. Eustis said the small number of tumors along with the lack of preneoplastic lesions did not support this being a chemically-related effort.

Dr. Garman moved that the Technical Report on polysorbate 80 be accepted with the revisions discussed and with the conclusions as written for male rats,

equivocal evidence of carcinogenic activity, and for female rats and male and female mice, no evidence of carcinogenic activity. Dr. Hayden seconded the motion, which was accepted unanimously with ten votes.

1,2,3-Trichloropropane. Dr. R.D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of 1,2,3-trichloropropane by discussing the uses, human exposure and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in mice and rats. The conclusions were that:

> Under the conditions of these 2-year gavage studies, there was clear evidence of carcinogenic activity of 1,2,3-trichloropropane in male F344/N rats receiving 3, 10, or 30 mg/kg based on increased incidences of squamous cell papillomas and carcinomas of the oral mucosa and forestomach, adenomas of the pancreas and kidney, and adenoma or carcinoma of the preputial gland. Carcinomas of the Zymbal's gland and adenocarcinomas of the intestine may have been related to chemical administration. There was clear evidence of carcinogenic activity of 1,2,3-trichloropropane in female F344/N rats receiving 3, 10, or 30 mg/kg based on increased incidences of squamous cell papillomas and carcinomas of the oral mucosa and forestomach, adenoma or carcinoma of the clitoral gland, and adenocarcinomas of the mammary gland. Carcinomas of the Zymbal's gland and adenocarcinomas of the intestine may have been related to chemical administration.
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> There was clear evidence of carcinogenic activity of 1,2,3-trichloropropane in male B6C3F1 mice receiving 6, 20, or 60 mg/kg based on increased incidences of squamous cell papillomas and carcinomas of the forestomach, hepatocellular adenomas or carcinomas of the liver, and Harderian gland adenomas. Squamous cell papillomas of the oral mucosa may have been related to chemical administration. There was clear evidence of carcinogenic activity of 1,2,3-trichloropropane in female B6C3F1 mice receiving 6, 20, or 60 mg/kg based on increased incidences of squamous cell papillomas and carcinomas of the forestomach, hepatocellular adenomas or carcinomas of the liver, Harderian gland adenomas, and uterine adenomas, adenocarcinomas, and stromal polyps.
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> There were chemical-related increased incidences in basal cell and squamous hyperplasia of the forestomach, acinar hyperplasia of the pancreas, renal tubular hyperplasia and nephropathy of the kidney, and preputial or clitoral gland hyperplasia in male and female rats. Increased incidences of squamous hyperplasia of the forestomach and eosinophilic foci of the liver in male and female mice were chemical related.

Dr. Goodman, a principal reviewer, agreed with the conclusions. He asked whether any of the clinical findings in male rats could have been due to the severe chemical-induced nephropathy. Dr. Irwin said that although the neoplastic response was quite strong, one could not unequivocally rule out a contribution by the nephropathy. Dr. Goodman commented on the four widely used in vitro tests for genetic toxicity, and noted the inability of three of the assays (viz., mutagenesis in mouse lymphoma cells and chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells) to augment the ability of mutagenesis in Salmonella typhimurium to predict carcinogenicity of chemicals in long-term rodent studies. Therefore, he thought presentation of data from these assays should be very limited in this and other reports. Dr. S. Eustis, NIEHS, responded that the staff would reconsider their approach to the genetic toxicology presentation and discussion in the reports.

Dr. McKnight, the second principal reviewer, agreed with the conclusions in principle. However, she said that Zymbal's gland tumors should be included as support for clear evidence in male and female rats, noting that these tumors occur with statistically significant trends in both sexes and the incidences in the high dose groups exceed the ranges observed in historical control groups for both sexes. Dr. McKnight said more information should be given for why gavage was used since occupational exposure mainly occurs by inhalation, and there is also potential for human exposure in drinking water and dermally. Dr. Irwin commented that due to the presence of 1,2,3-trichloropropane in ground and surface water as well as drinking water, the numbers of people exposed orally may exceed those exposed by any other route.

Dr. Zeise, the third principal reviewer, also agreed in principle with the conclusions. She supported Dr. McKnight's call to include Zymbal's gland tumors in rats under clear evidence, and proposed that oral cavity squamous cell papillomas be added to the evidence for male mice. Dr. J. Haseman, NIEHS, noted that the inclusion of oral cavity tumors as part of the evidence for carcinogenicity in the other three experimental groups added weight to the proposed association with chemical treatment for these uncommon tumors in male mice. Dr. Zeise argued that squamous cell papillomas or carcinomas of the skin and liver tumors in male rats as well as squamous cell carcinomas of the large intestine in female mice should be included in the conclusions as findings that "may have been related to chemical treatment." Dr. Eustis said that discussion of these tumors could be added to the results.

Mr. Beliczky stated that in view of the widespread human exposure in polymer manufacture and as a solvent for degreasing and paint stripping there needed to be more emphasis and information in the report on dermal exposure and absorption. Dr. Davis pursued the issue of how the route of administration is selected; i.e., was this the route of primary human exposure or was the gavage route chosen to maximize the ability to detect a carcinogenic response? Dr. Eustis said NTP takes into consideration the route of human exposure but cost also needs to be considered - two feed studies can be conducted for about the same cost as one inhalation study. Dr. R. Griesemer, NIEHS, added that the agency or party nominating a chemical for study may specify a particular route of exposure. In this case there was considerable ground water contamination and an interest in oral exposures from the start.

Dr. Goodman moved that the Technical Report on 1,2,3-trichloropropane be accepted with the revisions discussed and the conclusions as written for male and female rats and mice, clear evidence of carcinogenic activity. Mr. Beliczky seconded the motion. Dr. McKnight offered an amendment that Zymbal's gland tumors be added to the list of neoplasms on which the level of evidence is based in male and female rats. Dr. Davis seconded the amendment which was accepted by seven yes to three no votes (Bailey, Carlson, Garman). The original motion by Dr. Goodman was then accepted unanimously with ten votes.

SHORT-TERM TOXICITY STUDIES

Black Newsprint Inks. Dr. J.F. Mahler, NIEHS, introduced the short-term toxicity studies of black newsprint inks by reviewing the uses of and rationale for studying black newsprint inks, composition of the inks, methodology for dermal application of the inks and mineral oils, experimental design, and results. In 13-week studies, test chemicals were applied twice weekly at dose volumes of 20 microliters for mice and 50 microliters for rats. Treatment groups for rats consisted of letterpress ink mixture, offset ink mixture, printing ink mineral oil, USP mineral oil, and clipped, untreated controls. Groups of mice were administered each of the four individual lots of both letterpress and offset inks, the composite mixtures of each, and printing ink and USP mineral oils, in addition to clipped untreated controls. Compound-related effects were limited in rats to decreased body weight gains in females treated with letterpress ink mixture and ink mineral oil, and increased liver and kidney weights in both sexes exposed to USP mineral oil; there were no local toxic effects at the site of application. In mice, liver weights were increased in most ink and mineral oil treatment groups of both sexes. Dermal toxicity was evidenced by scaliness and irritation at the application site in several treatment groups and microscopically by epidermal hyperplasia and inflammation in all treatment groups. In summary, dermal exposure to black newsprint inks and mineral oils resulted in few systemic effects. Local effects were evident only in mice and were consistent with those of a primary cutaneous irritant.

Dr. Davis, a principal reviewer, said this was a well designed and very complex study that was concisely and clearly reported. He commented that the summary statement reports little if any toxicity beyond the site of application, yet female rats had decreased weight gains and decreased heart and lung weights with respect to controls. He wondered if this could be attributed to decreased feed consumption. Dr. Mahler agreed that the body weight decrements were substantial enough to be seen as evidence of toxicity and this would be noted.

Dr. Bailey, a second principal reviewer, considered this to be a well written report and the experiments well done. He said references to the work of Blackburn et. al. on the modified Ames assay should be cited since this assay can accurately differentiate between carcinogenic and non-carcinogenic mineral oils, and also provide highly reliable estimates of the relative potency of such materials in mouse skin painting bioassays. Dr. Mahler said there was a later reference cited where the modified Ames method was used; however, the Blackburn et.al. references also could be included. Dr. Bailey said mention was made that the carcinogenicity of a particular mineral oil is related to its refining history, with less refined oils having greater carcinogenic potential. Therefore, he thought it would be helpful to obtain the refining history for the ink oils used in the study. Dr. Mahler said they would try to get information on the refining history and include a table in the Appendix. Dr. Bailey also requested the inclusion of mutagenicity data on USP grade mineral oil (USPMO) and printing ink mineral oil (PIMO) in the Appendix. He wondered why the C3H mouse was used. Dr. Bucher commented that NIOSH, in suggesting use of the C3H mouse, had indicated they had a database on skin paint studies with that strain. Dr. J. Haartz, NIOSH, cautioned that this was not a large database, much of it consisting of studies involving asphalt fumes and fume fractions.

Mr. Beliczky said reference should be made to the fact that carbon black contains polycyclic aromatic hydrocarbons (PAHs), noting that the International Agency for Research on Cancer (IARC) lists some of these PAHs in oil as human skin carcinogens. Dr. Mahler said additional references could be added. Dr. J. Freeman, Exxon, seconded Dr. Bailey's request that information on the refining history of the oils and also the carbon blacks be given in the report.

Formic Acid. Dr. M. Thompson, NIEHS, introduced the short-term toxicity studies of formic acid by reviewing the natural occurrences and uses of formic acid, experimental design, and results. Two and 13-week toxicity studies of formic acid were conducted in male and female rats and mice by whole body inhalation exposure to formic acid vapors. In 2-week studies, doses ranged from 31 to 500 ppm and there were microscopic lesions in the respiratory and olfactory epithelia in rats and mice exposed to 62.5 ppm and higher concentrations. Lesions consisted of squamous metaplasia, necrosis, and inflammation. In 13-week studies, doses ranged from 8 to 128 ppm. Microscopic changes in rats and mice ranged from minimal to mild in severity and generally were limited to animals in the 128 ppm groups. Lesions consisted of squamous metaplasia and degeneration of the respiratory and olfactory epithelia, respectively. Hematologic and serum biochemical changes were minimal to mild and, generally consistent with hemoconcentration. Overall, effects of formic acid were consistent with those of irritant chemicals administered by inhalation exposure. The no-observed-adverse-effect level (NOAEL) for respiratory injury was 32 ppm in rats and mice. There was no significant evidence of systemic toxicity in these studies.

Dr. Carlson, a principal reviewer, thought this was a well-done study. He asked when after the end of the 2-week study was the blood pH determined, noting that acidosis is an important problem with the acute toxicity of methanol through its metabolism to formate. Dr. Thompson said the pH was determined the day after the last exposure. Dr. Carlson asked that a rationale be given for using the inhalation route. Dr. Thompson said that the primary reason that formic acid was nominated was because of its structural relationship to formaldehyde, and because inhalation is an important route of exposure for humans.

Dr. Klaassen, a second principal reviewer, also thought this was a well performed study. His main concern was that there was an over-emphasis in the report to indicate that rodent data on formic acid might not be applicable to humans. He would agree if significant systemic toxicity had been observed, but it was mainly local toxic effects which might be very relevant for humans. Dr. Thompson responded that the lack of an effect in rats may be related to their resistance to formate toxicity and that was the reason given for the emphasis. Dr. Klaassen agreed but said there needed to be clarification that the local toxic effects would be similar between rodents and humans.

Dr. Zeise questioned the NOAEL (32 ppm), noting a reported olfactory epithelial lesion in a male rat at 32 ppm in the 13-week study. Dr. M. Elwell, NIEHS, said the olfactory degeneration was a minimal change and it was difficult to say that it was a treatment effect.

Glyphosate. Dr. P.C. Chan, NIEHS, introduced the short-term toxicity studies of glyphosate by reviewing the uses and rationale for study, findings from chemical disposition studies, experimental design, and results. Chemical disposition studies showed that 20-30% of orally administered glyphosate was absorbed and excreted in the urine. In 13-week studies, groups of male and female rats and mice were administered glyphosate in the feed at doses up to 50,000 ppm. At doses of 25,000 and 50,000 ppm in the feed, glyphosate reduced body weight gain, caused cytoplasmic alteration and hypertrophy of salivary gland acinar cells, and elevated serum bile acids, alkaline phosphatase, and alanine aminotransferase activities, but, no histopathologic lesions were observed in the livers of rats and mice. The salivary gland effects of glyphosate were shown to be mediated through an adrenergic mechanism which could be blocked by the adrenergic antagonist, propranolol. The no-observed-adverse effect level (NOAEL) for the salivary gland lesion was 3125 ppm in the feed for mice, but the lesion was observed in all dose levels studied in rats.

Dr. Garman, a principal reviewer, said this was a thoroughly prepared and detailed report which did an excellent job of reviewing the background as well as the available literature on glyphosate. He thought inclusion of the isoproterenol/propranolol study was helpful in establishing a mechanism for the salivary gland alteration. He asked for clarification as to whether severity grades were based only on the parotid salivary gland or whether changes with in the submandibular gland were also taken into consideration. Dr. J. Mahler, NIEHS, said the severity grades reflect only the parotid gland lesions.

Dr. Goodman, a second principal reviewer, also thought this to be a well written report. He said the lack of reproductive toxicity was an important observation which should be included in the Abstract.

Scientific Presentations

Variability in Tumor Rates Among Control F344 Rats in NTP Studies: Dr. Joseph Haseman, NIEHS, began by stating that concurrent control tumor rates are preferred but historical control rates can be useful, especially with rare tumors and studies where there may be a marginal increase in tumors in a treated group compared with concurrent controls. He listed important potential sources of variability in control tumor rates (Attachment, p.2) noting that these factors may result in inter-laboratory variability and time-related trends in tumor occurrence. Dr. Haseman summarized the results from 88 studies in F344 rats which supported earlier findings that showed corn oil gavage increased pancreatic acinar cell tumor rates and decreased incidences of mononuclear cell leukemias in male rats relative to untreated or water gavage controls while having no effect in female rats (Attachment, pp.4-5). He then discussed the increases in tumor rates over time, from the early 1970s to the early 1980s, for leukemias and several other tumors, primarily of endocrine origin, in both sexes (Attachment, pp.6-7). Dr. Haseman further discussed trends in leukemia rates, and presented individual study rates to illustrate the variability in this tumor across control groups and the determination of a "normal" control range. (Attachment, pp. 9-10). He concluded his presentation with an example of inter-laboratory variability in control tumor rates (Attachment, p. 11).

Dr. Haseman said the evidence from corn oil gavage controls was quite convincing that the gavage technique, itself, had no effect in either sex of rats but rather, differences seen were due to the corn oil. Further, incidence rates for adrenal, pituitary and thyroid tumors seem to be leveling out in more recent data, while rates for mammary gland tumors and leukemia are still rising. The NTP is studying the role of dietary factors.

Mononuclear Cell Leukemia in the F344 Rat- Research Approaches: Dr. Richard Irwin, NIEHS, described the characteristics of mononuclear cell leukemia (MCL) in F344 rats, noting that MCL originates in the spleen, and the MCL cell which is a large granular lymphocyte. Although labeled a 'rat' disease, humans have large granular lymphocytes, and recently there have been about 50 cases cited in the literature of a chronic proliferative disease of these cells. He reiterated concerns expressed by Dr. Haseman about the reduced survival and high and variable incidences in controls which complicate interpretation of the effects of chemical exposure. Dr. Irwin discussed some research approaches by the NTP including: (1) evaluating the influence of diet composition; (2) the effects of chemical exposure, doing a retrospective examination of the NTP data base of studies employing the NIH-07 diet; (3) attempting to correlate structural/or metabolic properties of a chemical with pattern of leukemic response; and (4) examining the influence of toxic or neoplastic responses at other sites on the incidence of MCL. Goals of NTP studies are to: (1) reduce the incidence of MCL in F344 rats; (2) gain some insights into the mechanism of development of MCL; and (3) obtain a better understanding of changes in the incidence associated with chemical exposure.

Evaluation of the Usefulness of Interim Sacrifices in NTP Studies: Dr. Scot Eustis, NIEHS, stated that interim evaluations have been a standard part of NTP two-year studies, usually at 15 months with 10 animals per group, and including measurements of body and organ weights, hematology, clinical chemistry, and histopathology. Interim evaluations constitute about 10% of the cost of a feed

study so as part of an analysis of the cost-effectiveness of two-year studies, an examination was made of the their usefulness. He presented data from the first 13 studies with interim evaluations that had been peer reviewed by the Subcommittee. Conclusions drawn from this analysis were: (1) interim evaluations have not contributed to carcinogenicity determination; (2) they have not contributed substantively to clarifying or identifying chronic toxicity; (3) they have not contributed substantively to characterizing progression of lesions; (4) routine inclusion of interim evaluations in 2-year carcinogenicity studies is not cost effective; (5) they may be warranted when testing specific hypotheses in carcinogenicity studies; and (6) interim evaluations may be warranted in studies designed more specifically to determine chronic toxicity.