Board of Scientific Counselors
National Toxicology Program

Summary Minutes
from
Peer Review of Draft Technical Reports of Long-Term
Toxicology and Carcinogenesis Studies Including the Effects of Dietary
Restriction on Feed Studies and a Short-Term Toxicity Study
by the Technical Reports Review Subcommittee
and
NTP Toxicology and Collaborative Carbon Disulfide (CS_2) Inhalation Studies

on

June 20-21, 1995

Research Triangle Park, N.C.

The meeting began at 8:30 a.m. on June 20-21 in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee are: Drs. Arnold Brown (Chairperson), Thomas Goldsworthy, Meryl Karol, Curtis Klaassen, Claudia Miller, Janardan Reddy, Irma Russo, Louise Ryan, Robert Taylor, Mary Jo Vodicnik, and Jerrold Ward. These minutes have been reviewed and approved by all members of the Subcommittee. 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, N.C., 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 December 5 and 6, 1995, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, 919/541-3971.

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Butyl Benzyl Phthalate. Dr. F. W. Kari, NIEHS, introduced the toxicology and carcinogenesis studies of butyl benzyl phthalate (BBP) by describing the uses of the chemical and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic lesions in male rats and uncertain neoplastic findings in female rats as well as compound-related non-neoplastic lesions in male and female rats. Dr. Kari noted that there had been an earlier two-year NTP study with BBP in which there was no evidence of adverse effects in mice, there was a marginally increased incidence of mononuclear cell leukemia in female rats, and the study was inadequate in male rats due to high mortality beginning around week 14. Thus the design of the prechronic phase of the current study in male rats was more elaborate in an attempt to determine whether early mortality was likely or not to be a problem. The design included a 10-week modified mating trial as well as other indices of reproductive toxicity. The conclusions for the two-year studies were that:

> Under the conditions of this two-year feed study, there was **some evidence of carcinogenic activity** of butyl benzyl phthalate in male F344/N rats based on the increased incidences of pancreatic acinar cell adenoma and of acinar cell adenoma or carcinoma (combined). There was **equivocal evidence of carcinogenic activity** of butyl benzyl phthalate in female F344/N rats based on marginally increased incidences of pancreatic acinar cell adenoma and of transitional epithelial papilloma of the urinary bladder.
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> Exposure of rats to butyl benzyl phthalate in feed for two years resulted in focal hyperplasia in the pancreas in male rats and in transitional epithelial hyperplasia in the urinary bladder of female rats.

Dr. Reddy, a principal reviewer, agreed with the conclusions although he thought that the conclusions from the previous two-year study in mice should be cited. Dr. Kari responded that we would add this information to the Abstract, including why the current study was rats only and citing the findings. Dr. Reddy noted the 20% incidence of pancreatic acinar cell adenomas vs. only 2% incidence of carcinomas in high dose male rats. He asked for definition of the criteria for distinguishing adenomas from carcinomas since based on his extensive experience he wondered if carcinomas were not underrepresented. Dr. R. Hailey, NIEHS, said more would be added concerning how the distinction is made between adenomas and carcinomas. While metastasis is an easy marker for a carcinoma, other features are used such as cellular pleomorphism or tremendous heterogeneity in the growth pattern, cellular atypia, and high mitotic index.

Dr. Klaassen, the second principal reviewer, agreed with the conclusions. He said reference to the mouse studies should be made earlier in the report, preferably in the Abstract, and as well, in regard to the rationale, more information should be included on phthalate carcinogenicity. Dr. Kari said he would include more information on what is known about phthalate carcinogenicity. Because of the known effects of phthalates on male reproduction, Dr. Klaassen was pleased to see this studied here.

Dr. Ryan, the third principal reviewer, agreed with the conclusions. She wondered whether there should also be a conclusion regarding the reproductive toxicity. Dr. Ryan found it worrisome that so many animals died in the 26-week study during anesthesia prior to blood sampling and asked whether there was any bias here whereby weaker or sicker animals were more likely to die during the procedure. Dr. Kari said he could only speculate that the higher ratio of CO_2 to oxygen than generally used may have contributed to the excessive mortality. It did not appear to be due to chemical interaction in that the numbers of deaths in controls were similar to that in dosed animals.

Dr. Miller noted the NIOSH reference that over 300,000 workers are potentially exposed to BBP and asked whether there was data about occupational exposure in terms of airborne levels. Dr. J. Haartz, NIOSH, commented that their data base does not have such quantitative information. She reported that OSHA does have an exposure data base and offered to follow up on that for Dr. Kari.

Public Comment: Dr. M. Stevens, Manager of Toxicology Projects, Monsanto Business Services, stated that the biggest concern of Monsanto, a primary maker of BBP, was that this study not be considered in isolation from theirs and studies of others. He pointed out that survival and tumor findings in the earlier NTP study were not repeated in the current study, and further, when diet restriction was employed, the increased incidence of pancreatic tumors seen in the current study was eliminated. Thus, looking at the multiple studies with no consistent reported findings, he thought a decision could not be made about the potential carcinogenicity of BBP.

Dr. Miller asked the industry representatives for information on occupational and consumer exposures. Dr. R. Hogue, Monsanto, said occupational exposure is quite low as is consumer exposure in vinyl flooring because the BBP is bound into a polymeric system. He said exposure data would be provided to the NTP. Dr. R. Hart returned to the effects of feed restriction on tumor incidence, noting that in 30-month feed restricted male rats three exposed rats developed pancreatic acinar cell adenomas. In feed restricted dosed females at 30 months, there was a statistically increased incidence of neoplasms of the urinary bladder.

Dr. Ryan moved that the Technical Report on butyl benzyl phthalate be accepted with the revisions discussed and with the conclusions as written for male rats, **some evidence of carcinogenic activity**, and for female rats, **equivocal evidence of carcinogenic activity**. Dr. Reddy seconded the motion, which was accepted unanimously with ten votes.

t-Butylhydroquinone. Dr. K. M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of *t*-butylhydroquinone (TBHQ) by discussing the uses of the chemical 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 rats. The conclusions for the studies were that:

> Under the conditions of this long-term feed study, there was **no evidence of carcinogenic activity** of *t*-butylhydroquinone in male or female F344/N rats exposed to 1,250, 2,500, or 5,000 ppm. Under the conditions of this two-year feed study there was **no evidence of carcinogenic activity** of *t*-butylhydroquinone in male or female B6C3F1 mice exposed to 1,250, 2,500, or 5,000 ppm.
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> Exposure of male rats to *t*-butylhydroquinone in feed for a long-term period resulted in increased incidences of kidney cysts and inflammation.
>
> Exposure of rats to *t*-butylhydroquinone in feed for a long-term period resulted in decreased incidences of mammary gland neoplasms in males and females.

Dr. Vodicnik, a principal reviewer, agreed with the conclusions. She complimented the staff on the comprehensive review of the literature while recommending that a reference to a flawed study be deleted. Dr. Miller suggested the reference be kept but note the limitations of the study in the text.

Dr. Reddy, the second principal reviewer, agreed with the conclusions. He inquired as to whether the splenic pigmentation was hemosiderin or whether this could be the compound or lipofuscin. Dr. R. Hailey, NIEHS, said some stains for hemosiderin and perhaps, lipofuscin would be done. Dr. Reddy asked whether the nephropathy in male rats was associated with increased levels of $\alpha_2\mu$-globulin. Dr. Hailey responded that a minor contribution could not be ruled out absolutely, but there was no evidence in the subchronic or chronic studies that this protein played a significant role.

Dr. Miller, the third principal reviewer, agreed with the conclusions. She thought a side-by-side comparison of the TBHQ dose levels used in rats and mice and those found in a typical human diet would be useful for the reader. Dr. Abdo agreed to do so.

Public Comment: Dr. W. Faber, Eastman Chemical Company, commended the NTP on a well conducted study. He stated that the proposed effect of TBHQ on the male and female reproductive systems seemed rather tenuous given the lack of a clear dose-response relationship as well as lack of findings in the teratology and multigeneration studies. He said the mention of THBQ as being structurally related to hydroquinone and butylated hydroxytoluene which are described as carcinogenic chemicals should be clarified to indicate these chemicals are carcinogenic in experimental animals.

Dr. Miller moved that the Technical Report on *t*-butylhydroquinone 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. Reddy seconded the motion, which was accepted unanimously with ten votes.

Codeine. Dr. J. K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of codeine 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 mice. Dr. Dunnick also reported on toxicokinetic studies in rats and mice performed in collaboration with investigators at Burroughs Wellcome. The conclusions for the two-year studies in rats and mice were that:

> Under the conditions of these two-year feed studies, there was **no evidence of carcinogenic activity** of codeine in male or female F344/N rats exposed to 400, 800, or 1,600 ppm. There was **no evidence of carcinogenic activity** of codeine in male or female B6C3F$_1$ mice exposed to 750, 1,500, or 3,000 ppm.
>
> Thyroid gland follicular cell hyperplasia was increased in exposed male and female mice.
>
> Decreased incidences of benign pheochromocytoma of the adrenal medulla in male rats and mammary gland fibroadenoma and fibroadenoma or adenocarcinoma (combined) in female rats were related to codeine exposure.

Dr. Taylor, a principal reviewer, agreed with the conclusions. He observed that as noted in the Technical Report, the analgesic action of codeine depends on O-demethylation to morphine, a reaction that is mediated in humans by CYP P$_{450}$IID6, and he provided additional references. He said the discussion on human metabolism of codeine should be expanded. Dr. Dunnick said the additional references would be included.

Dr. Miller, the second principal reviewer, agreed with the conclusions. She said a side-by-side comparison of likely or usual human doses with doses used in the rodent studies would be helpful in the Abstract. Dr. Miller said some interpretation was needed on the significance of dose-related increases in sister chromatid exchanges occurring at concentration levels that caused cell cycle delay in Chinese hamster ovary cells. Dr. Dunnick said she would try to clarify these findings.

Dr. Vodicnik, the third principal reviewer, agreed with the conclusions. She said she was pleased at the inclusion of pharmacokinetic data and wondered whether there could not be a stand alone results section describing them. Dr. Vodicnik thought there might be too much emphasis in the references on studies with questionable design or interpretation, and in view of the large body of literature available these references could be deleted.

Dr. A. Turturro, NCTR, asked whether some quantification could be added relating decreases in body weight with decreases in mammary and adrenal tumors in rats. Dr. J. Haseman, NIEHS, responded that adrenal tumors were decreased at all three doses, including two that had no body weight effects. However, he said that decreases in mammary tumors could in part be explained by reduced body weight, and perhaps some quantification of the extent of this association could be added.

Dr. Vodicnik moved that the Technical Report on codeine be accepted with revisions discussed and with the conclusions as written for male and female rats and mice, **no evidence of carcinogenic activity**. Dr. Miller seconded the motion, which was accepted unanimously with nine votes.

1,2-Dihydro-2,2,4-Trimethylquinoline. Dr. R. D. Irwin, NIEHS, introduced the toxicology and carcinogenesis studies of 1,2-dihydro-2,2,4-trimethylquinoline (DHTMQ) by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting om compound-related neoplastic lesions in male rats and non-neoplastic lesions in male and female rats. The conclusions for the studies were that:

> Under the conditions of these two-year dermal studies, there was **some evidence of carcinogenic activity** of 1,2-dihydro-2,2,4-trimethylquinoline in male F344/N rats, based on increased incidences of renal tubule adenomas and adenomas or carcinomas (combined). There was **no evidence of carcinogenic activity** of 1,2-dihydro-2,2,4-trimethylquinoline in female F344/N rats receiving 36, 60, or 100 mg/kg, or in male or female B6C3F1 mice receiving 3.6, 6, or 10 mg/kg.
>
> Exposure of rats to 1,2-dihydro-2,2,4-trimethylquinoline by dermal application in acetone for two years resulted in acanthosis in males and females and hyperkeratosis in females at the site of the application. No nonneoplastic lesions in male or female mice were attributed to treatment with 1,2-dihydro-2,2,4-trimethylquinoline.

Dr. Irwin summarized the results of the 52-week initiation/promotion study in female SENCAR mice. He said that DHTMQ did not promote DMBA-initiated skin, while TPA did not promote DHTMQ initiated skin in SENCAR mice. Thus, in this system, DHTMQ did not behave as either an initiator or a promoter.

Dr. Karol, a principal reviewer, agreed with the conclusions. She noted that survival of both control and treated male rats was reduced from that of females by week 90 and said an explanation in the text was warranted.

Dr. Ryan, the second principal reviewer, did not fully agree with the conclusions in male mice where she would have supported **equivocal** or even **some evidence of carcinogenic activity**. She wondered if it were not premature to use the Seilkop method to discount the dose effect on liver tumors in male mice, since the procedure used to adjust for weight effects on tumor incidence may not be broadly enough accepted at this point. Dr. J. Haseman said that although Seilkop's logistic regression model was relatively new, it had provided an excellent fit to individual tumor and body weight data from over 3,500 animals in the NTP historical control data base. Dr. Ryan asked whether it would be possible to use a dose response model to decide on dose levels for the chronic study, based on the short-term studies, focusing her concern on why an intermediate dose between 50 and 100 mg/kg was not chosen as the top dose for female rats in the two-year study. Dr. Irwin responded that doses intermediate to those in the prechronic study are frequently picked. However, the consensus for this study was that the 100 mg/kg dose for rats did not produce a severe enough reaction on the skin to worry about in terms of the two-year study.

Dr. Ward, the third principal reviewer, agreed with the conclusions. He noted that the incidence rate for combined liver tumors in high dose male mice was above the historical control range for both feed and dermal studies, but agreed it was probably reasonable to discount them after adjusting for weight effects on tumor incidence. More information on how this was done would be helpful. Dr. Irwin said body weight was not the only factor but

also lack of nonneoplastic liver lesions in males and the lack of supporting neoplastic lesions in female mice entered into the interpretation. Dr. Ward said another discussion point was that the incidences of liver tumors were similar in high and low dose groups, and there was no increase in tumor multiplicity nor incidences of foci. Dr. Goldsworthy said he was unconvinced that increases in liver foci had been ruled out in male mice. Dr. M. Stevens, Monsanto, commented that in his experience with short-term and long-term studies with other quinolines, liver was the target organ.

Dr. Ward moved that the Technical Report on 1,2-dihydro-2,2,4-trimethylquinoline be accepted with the revisions discussed and with the conclusions as written for male rats, **some evidence of carcinogenic activity**, and for female rats and male and female mice, **no evidence of carcinogenic activity**. Dr. Miller seconded the motion, which was accepted with seven yes votes and one abstention (Goldsworthy). (Dr. Goldsworthy said he lacked enough information on the liver response in male mice to dismiss a higher level of evidence.)

Salicylazosulfapyridine. Dr. F. W. Kari, NIEHS, introduced the toxicology and carcinogenesis studies of salicylazosulfapyridine (SASP) by discussing the uses of SASP and rationale for study, 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. Dr. Kari reported that in 16-day and 13-week studies in male and female rats, there was gross and morphological evidence of thyroid gland hyperplasia that was confirmed by clinical chemistry indicative of a derangement of the pituitary-thyroid axis. As a result, stop studies were designed in male rats whereby they were exposed to the top dose of SASP for six months and then carried out for the remainder of the two-year term. The expected thyroid lesions were not seen in any of the animals carried to two years. The conclusions for the studies were that:

> Under the conditions of these two-year gavage studies, there was **some evidence of carcinogenic activity** of salicylazosulfapyridine in male and female F344/N rats based on increased incidences of neoplasms in the urinary tract. There was an increased incidence of transitional epithelial papilloma of the urinary bladder in males and a low incidence of rare transitional epithelial papillomas of the kidney and of the urinary bladder in females. Every animal that had a urinary bladder papilloma was observed to have bladder calculi.
>
> There was **clear evidence of carcinogenic activity** of salicylazosulfapyridine in male and female B6C3F1 mice based on increased incidences of hepatocellular neoplasms.
>
> Increased incidences of nonneoplastic lesions of the urinary bladder and kidney in male and female rats and of the spleen in male rats were observed. Increased incidences of nonneoplastic lesions of the liver and spleen in male and female rats were observed.
>
> Decreased incidences of mononuclear cell leukemia in male and female rats were related to salicylazosulfapyridine administration. Decreased incidences of forestomach squamous cell papilloma in female mice and forestomach hyperplasia in male and female mice were related to salicylazosulfapyridine administration.

Dr. Ward, a principal reviewer, agreed with the conclusions. He thought that male rats might have tolerated a higher dose. Dr. Kari agreed. Dr. Ward said the stop-exposure studies had limited significance in that neither preneoplastic or neoplastic lesions were shown to have occurred at 26 weeks. Dr. M. Elwell commented that based on the hyperplasia and thyroid effects at 13 weeks, it was reasonable to expect that they would be present at 26 weeks.

Dr. Goldsworthy, the second principal reviewer, agreed with the conclusions, but had concerns with dose selection in the mice. Given that liver weight changes and centrilobular hypertrophy were observed in all doses in 13-week studies, he questioned why there wasn't a dose chosen for the 2-year studies where changes had not been seen. Dr. Elwell said that at the low dose in 13-week studies hypertrophy could not be detected morphologically in female mice and there was only a 10-15 % increase in liver weight in males. Dr. Goldsworthy commented that the same rationale given for stop-studies in rats could have been applied to the mouse and mouse liver tumors. Dr. Kari reported that at the time the studies were designed the thyroid gland hyperplasia appeared to be the predominant effects

from SASP, and further, there was great interest in the role of endocrine disruption by goitrogenic compounds, such as the aryl sulfonamides, being associated with thyroid neoplasia.

Dr. Russo, the third principal reviewer, agreed with the conclusions. She noted that review of the literature indicates probability of fetal damage in humans from SASP, as well as transplacental transport and secretion in milk. Lack of data on absorption and disposition of SASP in pregnant experimental animals suggests future studies of transplacental effects would be desirable.

There ensued a discussion about the renal tumors in rats and the association with bladder calculi (concretions). Dr. J. Bucher, NIEHS, noted that the fact that every animal with a bladder or kidney papilloma also had grossly observable calculi strongly supported an association. Dr. Reddy inquired as to the nature of the concretions. Dr. Kari responded that they were spiculated in nature but were not chemically analyzed. The calculi were presumed to be precipitated drug and/or metabolites. Dr. Russo asked how the findings could be extrapolated to humans when the doses in animals were so much greater. Dr. Kari said the experiments in rodents are designed to maximize the probability for identifying the potential for toxicity and carcinogenicity. He pointed out that discussion of our toxicokinetics evaluation in the report compares blood levels reported after maintenance doses in humans with blood levels in rodents used in these studies. At least for some of the doses, there were comparable blood levels observed between the species.

Public Comment: Dr. A. Imondi, Director, Toxicology and Safety Assessment, Pharmacia, stated that after many years of extensive clinical use as a therapeutic agent in inflammatory bowel disease and rheumatoid arthritis there is no evidence that SASP is tumorigenic in humans. With regard to positive micronucleus tests observed in mice by the NTP, he cited unpublished studies that indicated folic acid deficiency was involved and increased incidences of micronuclei could be reduced or reversed by folic acid supplements with drug treatment. Dr. Imondi described other unpublished studies showing SASP treatment did not produce increases in DNA adduct formation in target tissues. Thus, he concluded that the NTP tumor findings along with clinical experience indicated no relevance for humans.

Dr. Ward moved that the Technical Report on salicylazosulfapyridine be accepted with the revisions discussed and with the conclusions as written for male and female rats, **some evidence of carcinogenic activity**, and for male and female mice, **clear evidence of carcinogenic activity**. Dr. Russo seconded the motion, which was accepted unanimously with ten votes.

Scopolamine Hydrobromide Trihydrate. Dr. K. M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of scopolamine hydrobromide trihydrate by discussing the uses of the chemical and rationale for study, describing the experimental design, and reporting on survival and body weight effects. In the 14-week study in rats, there was considerable mortality at higher doses attributed to esophageal and tracheal obstruction by feed and bedding, a condition considered secondary to inhibitory effects of scopolamine on salivary secretion and the motility of smooth muscle involved in swallowing. The conclusions for the two-year studies were that:

> Under the conditions of these two-year gavage studies, there was **no evidence of carcinogenic activity** of scopolamine hydrobromide trihydrate in male or female F344/N rats or B6C3F1 mice administered 1, 5, or 25 mg/kg.

Dr. Klaassen, a principal reviewer, agreed with the conclusions. He said that he was surprised that more scopolamine could be administered by gavage than in the feed and asked for elaboration. Dr. Abdo explained that the problem with esophageal obstruction even at lower doses diminished the amount that could be administered in the feed. Dr. Klaassen noted that a decrease in body weight was often given as an explanation for a decrease in tumors such as reported here, and inquired as to creation of a graph to let the reader evaluate this conclusion. Dr. J. Haseman, NIEHS, agreed that this was a good idea in concept, and he would evaluate two potential approaches to illustrate this association, one using a model based on logistic regression to predict tumor incidence for animals at a particular body weight, and the other looking empirically at a moving average of tumor incidence for animals in the data base of a given weight range.

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

Dr. Taylor, the third principal reviewer, agreed with the conclusions. He and Dr. Russo were complimentary of the inclusion of data allowing evaluation of neurobehavioral toxicity, and also the inclusion of pharmacokinetic studies enabling correlation of toxic effects with plasma levels. Dr. Taylor questioned listing all of the therapeutic uses associated with scopolamine as in his experience the drug had not been employed for a number of the listed conditions during the last 20 years. Dr. Abdo said he would tighten up the wording by using past tense for therapeutic uses that no longer apply.

Dr. R. Hart observed that body weight reductions may have a sparing effect relative to neurobehavior and other neurological endpoints and said he would supply Dr. Abdo with references. Dr. Goldsworthy strongly supported inclusion of pharmacokinetic studies, particularly encouraging their use in more of a prospective fashion to help set dose levels enabling better comparisons across species. Dr. G. Lucier, NIEHS, agreed and said that within the NIEHS an interdisciplinary toxicokinetics faculty has been established which serves to help determine the kinds of specific studies that should be incorporated into the design for a chemical. In some cases the data obtained would be such as to aid in development of a biologically based dose-response model. Dr. Miller said it would be useful to include in the section on production and use and human exposure actual drug doses commonly used in medical practice including average duration of treatment.

Dr. Klaassen moved that the Technical Report on scopolamine hydrobromide trihydrate 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. Taylor seconded the motion, which was accepted unanimously with ten votes.

Effect of Dietary Restriction on Toxicology and Carcinogenesis Studies in F344/N Rats and B6C3F1 Mice. Dr. F. W. Kari, NIEHS, introduced the studies on the effect of dietary restriction (DR) on toxicity and carcinogenicity of selected chemicals in rodents by noting that it has been recognized since the turn of the century that body weight reductions or feed restriction with concomitant decreases in body weight results in increased longevity and decreased incidences of a variety of neoplastic and nonneoplastic lesions. He showed an abbreviated list of literature reports indicating this phenomenon is not unique to a particular species, strain, sex, tumor site, or carcinogen. Dr. Kari said the two primary objectives were: (1) to evaluate the effect of moderate body weight reductions, nominally 15 %, on sensitivity of NTP bioassays; and (2) to evaluate the effect of weight-matched control group inclusion on the sensitivity of NTP bioassays. He then reviewed the overall design of the studies with the four chemicals: butyl benzyl phthalate; *t*-butylhydroquinone; salicylazosulfapyridine; and scopolamine hydrobromide trihydrate. Dr. Kari described the four basic comparisons, being: (1) the top dose of the 'standard' bioassay with controls, both fed *ad libitum* (*ad lib*) (the bioassays with all dose groups had been reviewed by the Subcommittee); (2) the same top dose group compared with weight matched controls; (3) feed restricted control and top dose groups; and (4) feed restricted control and top dose groups allowed to go on for three years or to 20% survival. In six of the eight experimental groups, the body weights of the weight-matched controls came within a percent or two of the body weights of the top-dosed animals.

Dr. Kari provided an overview of the results, looking for discordance or disparities between the outcomes under the various protocols. Overall, there were nine cases in which a statistically or biologically significant increase in some neoplastic finding was observed relative to the control. Specifically, a comparison of control and top dose animals under *ad lib* conditions (reviewed in the chemical-specific reports) indicated statistically significant increases in three sites, that is, butyl benzyl phthalate - pancreas in male rats; and salicylazosulfapyridine - urinary bladder in male rats and liver in male mice. In contrast, none of these three tumor sites were statistically significant when evaluated under the feed restricted protocol. However, using the pair weighted controls, the three lesions seen under *ad lib* conditions were significantly increased, as were two lesions seen after three years of feed restriction: *t*-butylhydroquinone (clitoral gland tumors in female rats), and butyl benzyl phthalate (urinary bladder tumors in female rats). In addition, using weight-matched controls, four other tumor increases were found to be statistically significant: butyl benzyl phthalate - mononuclear cell leukemia in male and female rats and adrenal pheochromocytomas in male rats; and *t*-butylhydroquinone - preputial gland tumors. Dr. Kari proceeded to discuss tumor sites particularly with regard to biological plausability of weight reduction vs. chemical exposure as determinants of incidence for certain tumors.

Dr. Kari summarized the findings: (1) two of the four chemicals caused increased incidences of neoplasms at three sites under the *ad lib* protocol for 104 weeks; (2) none of these three sites were identified as targets under the feed restricted protocol; and (3) the magnitude of the tumor responses was greater when the weight-matched protocol was used. He concluded that: (1) the sensitivity of the bioassay to detect carcinogenic responses was altered by dietary restriction; and (2) the association between reduced body weights and decreased neoplasm incidences underlie the necessity that doses not exceed "minimally toxic doses"; such body weight changes complicate the detection of carcinogens.

Principal Reviewers' Comments:
Dr. Goldsworthy, a principal reviewer, stated that the experimental results were predictable, given the preexisting literature and especially the limited responses seen with the four chemicals. The study did confirm what has been known, that increased survival and decreased observance of certain neoplasms occur in dietary restricted studies. His major criticism was that selection of the chemicals limited the number of insights and conclusions that could be made. He said that chemical selection should have included both weak and strong carcinogens and they should have been chosen ideally to examine tissues that are sensitive to dietary restriction as well as targeting low spontaneous tumor sites where normally diet restriction (DR) changes in untreated animals could not be observed. He said this was not the case, and it is not clear how the chemicals were chosen. Dr. Goldsworthy pointed out that the studies were properly conducted and thought that some of the interesting insights of the study were in examining the limited responses or subtle differences that did occur. This is important because there is a need both in the literature and in future studies for teasing out DR effects on very small and variable changes after long-term chemical administration.

Dr. Weindruch said he would preface his comments to say that they should be viewed as those of a gerontologist with a long term interest in the retardation of aging and diseases by dietary restriction. His main scientific concern involved the lack of precise definition of the *ad lib* food intake, and the methods described did not lend confidence that this was precisely measured. In his experience with many strains of rats and mice, *ad lib* intake displayed considerable animal to animal variation. Thus, within a target of 15%, there would be a very large range, and the use of group housing added to this problem. Dr. Weindruch spoke against the stated and naive implication that dietary restriction 'works' by preventing obesity, and spoke for diets enriched in vitamins, minerals, amino acids, so as to balance the intakes of dietary essentials among rodents fed different levels of calorie intakes and undergoing toxicology testing. Finally, he stated that the scientific rationale for choosing the test chemicals needs to be stated clearly in a prominent place, and why particular doses and routes of administration were selected.

Dr. R. Hart said his foremost criticism had to do with the choice of compounds, commenting that if he were going to test a new paradigm for conducting bioassays, he would not choose randomly four chemicals for evaluation. Dr. Hart commented that use of a Maximum Tolerated Dose (MTD) derived from an *ad lib* fed animal to calculate dose in DR fed animals is misleading at best, as toxic endpoints can be more severely impacted than carcinogenicity. He said that weight restricted controls fail to take into consideration the impact that switching caloric intake can have on a number of key physiological, metabolic, biochemical, and molecular parameters, e.g., polydipsia, increased renal clearance, or alteration of key drug metabolizing enzymes in DR animals. Dr. Hart found disconcerting a perceived lack of concern by the investigators that the data, in his view, fly in the face of 50 years of similar studies, conducted in over 20 laboratories, using over 30 different model carcinogens, which have shown that in general DR delays the onset or reduces the severity of tumor endpoints but does not completely eliminate such endpoints. It was also important to note that where chemically-induced tumor endpoints appeared to be eliminated in the current studies, the mean weights of the dosed groups were significantly less than those of the corresponding DR control groups. This compromised the assumptions that the tumor endpoints had really been eliminated. Dr. Hart stated that his main point, and the report's main point as he

viewed it, was that if DR is used it should be moderate, and to enhance interstudy reproducibility a more physiological normalizer, such as adjusting dietary intake to achieve an idealized weight curve, will be needed. He proposed that a small workshop be convened to discuss and decide what is an idealized weight curve, how it could be achieved, and how it could be monitored. The findings and recommendations could be reported back to the Board.

Program Response:
Dr. Kari acknowledged the suggestions concerned with using more idealized conditions. However, he stated that the primary purpose of the DR studies was to create a data base that would help both to clarify results retrospectively where there were alterations in body weight presumably due to primary or secondary effects of the chemical and to guide us in how to interpret prospective studies where alterations in body weight are expected. Thus, the experimental conditions should somewhat mimic those used in the bioassays, such as group housing and standardized diet. He said there was a definite lack of consensus in the large body of literature as to the best experimental conditions. It was important that we have a data base which allows us to make interpretations about often very subtle effects. With regard to the chemicals selected for study, Dr. Kari said selection was based in part on neoplastic and nonneoplastic lesions anticipated to come from the particular chemicals selected given the knowledge we had at that time.

Discussion:
Dr. R. Hart noted that the fact that the DR paradigm works under so many diverse conditions suggests it is a process we have to be very cognizant of in trying to make an evaluation of toxicity. He thought the NTP study could serve as a good baseline; however, we need better model compounds to test the paradigm. Dr. Weindruch said the driving force is the caloric intake *per se*. Dr. Karol opined that it was important that we look at mechanisms of effects seen in DR studies.

Public Comment:
Dr. Kevin Keenan, Merck Research Laboratories, said his laboratory was already using dietary restriction, although they called it proper nutrition in their studies with Sprague-Dawley rats. He said the per cent restriction is irrelevant but what is important is kilocalories/day/rat and showed data from studies in his laboratory and the Wistar Institute correlating Kcal/day with per cent tumors and tumors/rat. He opined that *ad lib* feeding is one of the most adverse events an animal can be subjected to. Dr. Keenan concluded by summarizing the positive and lack of adverse effects of moderate dietary restriction in their laboratories on animal health, longevity, and spontaneous and chemically-induced tumor incidences.

Concluding Discussion:
Dr. Miller stated that in view of the complexities, she supported bringing together experts in nutrition, geriatrics and toxicology to focus on the issues around dietary restriction and toxicology studies. Dr. G. Lucier, NIEHS, agreed it would be a good idea for the NTP to sponsor some sort of workshop, with the exact scope needing to be worked out, to address these issues. The findings and recommendations could be commented on in an open meeting, perhaps through the NTP Board. Certainly, chemical selection would be an important issue. Dr. J. Bucher, NIEHS, commented on the increasing body weights in

Fischer rats and the debate about whether the NTP will have to go to a more expensive and technically difficult dietary restriction regimen for all its studies. Dr. G. N. Rao, NIEHS, said that the key to stopping or reversing the upward drift of animal body weights is to go back to the production colonies or to establish your own colony to effect controls over growth patterns. Dr. A. Turturro, NCTR, observed that breeding back will not necessarily give the same animal. He said we must somehow control or reduce the tremendous individual animal variability within studies. Dr. W. Allaben, NCTR, reported that as an outcome of a conference last year, the FDA had put together a draft white paper looking at the issue of diet, variability of test outcomes, and the value of utilizing caloric restriction to try to control for that variability, and this document will go out soon for public comment. Dr. Kari said it was important that we don't mask false negatives or false positives. Returning to the concept of a workshop, Dr. Lucier commented that dietary restriction and effects on other toxicologic endpoints need to be addressed. Dr. R. Hart said that dietary control as a term might be preferable to dietary restriction. He said the FDA would cosponsor a workshop, while Dr. Karol indicated the Society of Toxicology would be interested in serving as a cosponsor. Dr. Keenan said the Society of Toxicologic Pathologists has plans for a symposium in June 1996, and suggested there needed to better integration among sponsoring groups.

Short-Term Toxicity Study

1,4-Butanediol. Dr. R. D. Irwin, NIEHS, introduced the NTP Summary Report on the Metabolism, Disposition, Toxicity, and Carcinogenicity of 1,4-Butanediol by reviewing the uses and rationale for study. He reported that there is significant literature on this compound which indicates that it is well absorbed and rapidly metabolized to gamma-hydroxybutyric acid, a naturally occurring chemical which is metabolized to CO_2 via the Tricarboxylic Acid Cycle. The report is largely a review of this literature indicating that toxicologic and pharmacologic responses to 1,4-butanediol are due primarily to its conversion to gamma-hydroxybutyric acid. Dr. Irwin said that a few years ago the NTP had conducted prechronic and 2-year studies on gamma-butyrolactone, which is also rapidly metabolized to gamma-hydroxybutyric acid, and demonstrated a lack of toxicologic and carcinogenic potential. Additionally, an NTP disposition study using radiolabelled 1,4-butanediol was included in the current report confirming the previous literature findings. Thus, Dr. Irwin concluded that there is a high likelihood that 1,4-butanediol would not be carcinogenic if long-term studies were conducted, and no further evaluation is needed. [Ed. Note - This is the second NTP 'prediction' report to come before the Subcommittee. Under ground rules approved unanimously by the Subcommittee in 1994, prediction reports should undergo mail review by one member and two *ad hoc* experts prior to concluding discussion by the Subcommittee as to whether or not to accept the recommendations of the reviewers.]

Dr. Vodicnik, the primary reviewer from the Subcommittee, said her comments would reflect her own review, as well those of the two *ad hoc* reviewers. She stated that as indicated by Dr. Irwin, 1,4-butanediol is rapidly converted to gamma-hydroxybutyric acid in all species studied, including humans. Following intravenous (iv) injection in humans, the plasma concentration-time profile of the metabolite is nearly superimposable over that obtained after iv injection of gamma-hydroxybutyric acid, demonstrating rapid and complete conversion. The nature and time course of pharmacological/toxicological responses to 1,4-butanediol reflect those of gamma-hydroxybutyric acid and antagonism of gamma-hydroxybutyric acid-induced sleep induction shortened sleeping time induced by 1,4-butanediol. Also, a structural analog, 1,3-butanediol, has been evaluated for chronic toxicity with no adverse effects observed. Dr. Vodicnik concluded that the data presented strongly support the pharmacologic and toxicologic responses to these chemicals being due to conversion to gamma-hydroxybutyric acid.

Dr. Vodicnik commented that the three reviewers agreed that the final sentence of the Conclusion should be reworded from "1,4-butanediol should be considered not carcinogenic in animals" to "1,4-butanediol should be considered unlikely to be carcinogenic in animals" since actual carcinogenicity or noncarcinogenicity cannot be assessed without running a bioassay. Dr. Karol said she strongly supported that change in wording. Dr. Ward said such wording should be used in future reports where a similar prediction is made. Dr. Goldsworthy added that the title of this report should not have "Carcinogenicity" included. Dr. Brown said there appeared to be a consensus among the members that with the changes suggested the Technical Report was acceptable.

Update on the New Diet (NTP-2000) for Rats and Mice in NTP Toxicology and Carcinogenesis Studies

Dr. G. N. Rao, NIEHS, reviewed the composition of the NIH-07 diet which had been the standard diet for NTP studies since 1980, noting that it was designed for production colonies and contained high protein (~23%) and vitamin D levels. The process for modifying this diet began about seven years ago and culminated with the adoption of the NTP-2000 open formula nonpurified diet which is the selected diet for all new NTP toxicology and carcinogenesis studies as of January 1995. the new diet contains lower protein (~14.5%) to decrease the severity of nephropathy while being adequate for growth and maintenance. The fat and fiber concentrations of NTP-2000 diet are higher than in the NIH-07 because various experimental diets evaluated during the past seven years indicated that these changes in diet could slow the body weight gain, delay the development of some spontaneous lesions and increase rodent survival in two-year studies. Dr. Rao said that in Fischer rats increasing fat appears to decrease the incidence of severity of leukemia or associated mortality, and increasing fiber, used to decrease caloric content, also had a beneficial effect in delaying development of mammary tumors. And a combination of either higher fat or fiber is decreasing the incidence of adrenal pheochromocytomas in male and to some extent in female rats. He reviewed the nutrient composition and rationale for levels chosen, and described a 90-day study with both diets to assess among other things, palatability. The study showed the new diet was indeed palatable. In conclusion, Dr. Rao said the NTP-2000 diet is adequate for growth and maintenance but may not be adequate for reproduction and lactation. The new diet appears to decrease the severity or delay the development of diet and age associated lesions. He said this diet may not be appropriate for studies involving dietary restriction.

Dr. Ward congratulated Dr. Rao on this effort and wondered if the tumor incidence data base will change quite a bit. Dr. Rao said he did not expect a drastic change but did expect definite improvements in health through a decrease in the chronic age associated lesions of rats and mice. Dr. Rao said he was now proposing that diet and bedding used in NTP studies be irradiated. He said recent increased incidences of *Helicobacter* infections suggested there may be some contamination of feed and bedding with opportunistic pathogens that irradiation might eliminate. Dr. Ward said he was not convinced that there was evidence supporting the need. Dr. Rao reported that molds are complicating some of our studies, as seen in nasal lesions, that come from either bedding or feed. Heat treatment used does not inactivate the mold spores while irradiation is supposed to eliminate these spores.

NTP Toxicology and Collaborative Carbon Disulfide (CS2) Inhalation Studies

Introduction — Dr. John Bucher, NIEHS, said that the Subcommittee would be given an extensive update on recently completed and still ongoing studies on carbon disulfide. The outcomes of the collected studies, when completed, will be published in peer reviewed journals. Dr. Robert Sills, NIEHS and study coordinator, said the carbon disulfide (CS_2) research team would present their findings from the neurotoxicity and atherosclerosis inhalation exposure studies. These studies resulted from a true partnership among scientists from the Environmental Protection Agency (EPA), Duke University Medical Center (Duke), and the NIEHS. He noted that the CS_2 research plan was approved by the NTP Board of Scientific Counselors in May 1993, and studies began in February 1994. He reviewed the uses and said the most profound effects in humans associated with chronic low exposures include peripheral neuropathies and atherosclerosis. The rationale for studying CS_2 is based on its high production volume, potential for human exposure, and inclusion under the Clean Air Act. In planning for studies, the NTP held a series of meetings with scientists from government, academia and industry. In the next phase, a decision will be made about performing developmental toxicity and chronic studies.

Dr. Sills reported that CS_2 belongs to a large group of environmental chemicals which cause central and peripheral neuropathies, and appears to act on distal portions of long axons in the central and peripheral nervous systems causing axonal swellings with accumulations of neurofilament proteins following which degeneration occurs distal to the swelling. Overall goals were to examine the progression and mechanism of CS_2 toxicity as a model for other environmental chemicals which may cause central or peripheral neuropathies, and provide critical information on dose-response relationships for a number of neurological endpoints. Dr. Sills reviewed the metabolism of CS_2 noting that dithiocarbamate derivatives may play a role in the development of distal axonopathy through covalent crosslinking of proteins. He concluded by listing the specific objectives: (1) to determine plasma concentrations of CS_2; (2) to measure crosslinking of spectrin in red cells and levels of the cyclic metabolite TTCA in urine as potential biomarkers of exposure; (3) to examine the potential molecular mechanism of the distal axonopathy; (4) to measure nerve growth factor receptor gene expression as an indicator of early peripheral nerve cell injury; (5) to characterize the morphological progression of the distal axonopathy; (6) to examine functional alterations by measuring electrophysiological endpoints; and (7) to examine behavioral changes with a functional observational battery (FOB).

In-Life Evaluations

Experimental Design and Toxicokinetic Studies — Dr. Daniel Morgan, NIEHS, described the study design and exposure and monitoring systems for inhalation exposures of male and female F344 rats to CS_2, noting that all endpoints were measured on the same animals. Rats were exposed to 0, 50, 500, or 800 ppm for 2, 4, 8, or 13 weeks by whole-body exposure, except for the next to last exposures where each group was nose-only exposed during collection of blood samples for toxcokinetic exposures. Body weights and clinical observations were collected weekly while all other collections and measurements were staggered over the last three exposure days such that collection of one endpoint did not affect subsequent endpoints.

Dr. Morgan said that overall the objectives of toxicokinetic studies were to estimate internal dose of CS_2 by characterizing kinetics of uptake and elimination after single and repeated exposures, and to evaluate the amount of CS_2 in blood and urinary levels of TTCA as biomarkers of exposure. The studies showed CS_2 to have a relatively high volume of distribution, likely due to high lipid solubility, and found that CS_2 is rapidly eliminated from blood on cessation of exposure, and both urinary TTCA and levels of CS_2 in the blood appeared to be specific for determining the exposures to CS_2.

Molecular Evaluations

Crosslinking of Spectrin and Neurofilament Proteins — Dr. Bill Valentine, Duke, said there had been interest in examining the potential for CS_2 to covalently crosslink proteins for two reasons, one being that crosslinking in a readily obtained protein in red blood cells could serve as a biomarker of exposure, and secondly, covalent crosslinking in neurofilaments could be an underlying mechanism for the accumulation of neurofilaments that is a characteristic lesion in CS_2-induced neuropathy. He described studies with spectrin found in erythrocyte membranes showing crosslinking in the form of a spectrin dimer. Dr. Valentine said they were able to demonstrate a cumulative dose response up to the limit of the lifetime of the red cell and were able to detect spectrin dimer formation at nonneurotoxic levels and prior to formation of neurofilamentous swelling. He also concluded that spectrin crosslinking had excellent potential as a biomarker for evaluating cumulative low-level exposure to CS_2. Dr. Valentine described studies showing that CS_2 covalently crosslinks neurofilaments and precedes detection of the neurofilamentous swelling and crosslinking is detectable at nonneurotoxic levels of exposure.

Peripheral Nervous System (PNS) Gene Expression (NGF-R) — Dr. Jean Harry, NIEHS, said the hypothesis they were testing was that exposure to CS_2 would increase the expression of low affinity nerve growth factor receptor, either within the Schwann cell or in the underlying axon, and that alterations within this gene would be expressed prior to any morphological changes observed. She discussed their studies with the sciatic nerve which suggested changes in the integrity between the axon and its myelin sheath related to a signaling mechanism in a portion of the nerve where there were no morphological changes occurring. There appeared to be metabolic perturbations within the Schwann cells marked by an increase in rough endoplasmic reticulum, mitochondria, gogi apparatus, and ribosomes.

Structural Evaluation

Morphological Studies — Dr. Sills stated that the objectives were to examine the progression and dose-response relationship of the distal axonopathy by light microscopy, and to characterize the axonopathy by electron microscopy and in teased nerve fiber preparations. To evaluate the central nervous system, the distal long axons in the white matter of the most proximal and distal portions of spinal cord, including cervical and lumbar segments, were examined, and to evaluate the PNS, the posterior tibial nerve was studied. The morphological studies were the first to illustrate the morphological progression and dose response of CS_2 distal axonopathy at time points as early as two, four and eight weeks in the spinal cord and tibial nerve. Axonal swelling was first present in the spinal cord at eight weeks and at 500 ppm and above, while in the tibial nerve, it was present only at 800 ppm. Degeneration often accompanied by regeneration was first detected at thirteen weeks

and at 800 ppm. Complex neurofilament accumulations were first detected microscopically at eight weeks, supporting the findings of crosslinking of neurofilament proteins in axons. Previous studies on spinal cord axonal swelling were extended with our studies showing prominent axonal swelling in the lateral and ventral funiculus nerve tracts. The long distal axons in the lumbar spinal cord segments were the most sensitive to CS_2 toxicity. In response to a query, Dr. Sills said demyelination was not seen but rather thinning of the myelin sheath.

Functional Evaluations

Electrophysiology Studies — Dr. David Herr, EPA, reported that the objective was to examine and quantify dose- and time-related changes, and nerve conduction velocity (NCV) and compound nerve action potentials (CNAP) produced by CS_2 in the ventral caudal tail nerve of rats. The studies demonstrated a slight decrease in NCV after 13 weeks exposure to 800 ppm, and this was not due to altered tail temperatures. Amplitudes were slightly increased after 13 weeks exposure to 500 or 800 ppm CS_2, and this effect again was not related to either tail temperature or diameter. The CNAP duration was slightly decreased after eight or 13 weeks exposures to 500 or 800 ppm, and the effect again was not related to either tail temperature or tail diameter.

Behavioral Studies — Dr. Ginger Moser, EPA, said objectives were to establish a profile of the functional changes produced by CS_2 and to characterize that profile in terms of the exposure concentration and duration. They eventually hoped to correlate behavioral changes with other endpoints since all measures were taken on the same rats. The functional observational battery (FOB) was used. The FOB can evaluate neuromuscular dysfunction, gross sensory deficits, changes in the autonomic nervous system or changes in activity or excitability states of the rats. The studies showed that CS_2 produced a clear profile of neuromuscular changes including gait and postural abnormalities, decreased grip strength, and ataxia. Hind limbs were preferentially affected over forelimbs. Other effects at 13 weeks included depressed reactivity to visual stimuli, loss of the pupillary reflex, and mild tremors. Shorter exposure lengths did produce changes in general excitability and reactivity, but this seemed to be an early effect which dissipated with longer exposures. In conclusion, CS_2 motor syndrome was related to exposure concentration and increased with the longer exposure lengths. Dr. Moser commented that these functional profiles will help in determining mechanisms of action for CS_2 as well as for other peripheral nerve toxicants.

Summary of CS2 Neurotoxicity Studies

NTP/NIEHS Perspective — Dr. Harry emphasized the value of the team approach in these studies, noting that there are other studies we may want to add using the tissues that are still available. She stressed the economy of effort enabled by having the investigators all nearby and the flexibility allowed by having the inhalation facilities relatively on site. Dr. Harry summarized the time/dose effects at the high dose (800 ppm). At two weeks, there are cellular changes as evidenced by crosslinking and functional changes as seen in alteration of gait, while at four weeks, increases were shown in a marker that may detect alterations in integrity of the axon and Schwann cell myelin sheath and there was progression in functional alterations. At eight weeks, axonal swelling can be detected, which is reversible on cessation of exposure. At 13 weeks, both axonal degeneration and

regeneration are seen as well as changes in the compound nerve action potential. Dr. Harry noted that 50 ppm had been chosen as the no effect level based upon a study by CIIT; yet in the current study, spectrin crosslinking and changes in gait were observed at that dose level. This raises the question as to what do we define as a neurotoxic event, an issue of some importance to NIEHS and the regulatory agencies. Do we not call anything neurotoxic until there is a functional change in the way an animal can either interact with its environment or respond to its environment, or do we go back and look at the molecular events and at what point do we call each of these components a neurotoxic event?

Biology and Mechanistic Perspective — Dr. Doyle Graham, Duke, underscored the value of the collaboration in allowing much more to be learned about the biological basis of CS_2 neurotoxicity than could have been achieved working separately. He thought that crosslinking of erythrocyte spectrin has potential to be a biomarker of cumulative effect. The crosslinking of spectrin does parallel that in the neurofilaments allowing this biomarker to be a direct biologic parallel to the molecular events occurring within the axon. Dr. Graham followed up on Dr. Harry's question as to what constitutes a neurotoxic event for purposes of regulation. Having the ability to detect cellular and molecular changes at lower dose levels challenges us to rethink the whole concept of NOAELs and LOAELs and consider what better models may be appropriate for regulatory decisions. He said that in the future because of the time course for axonal and other effects, longer periods of exposure for neurotoxic endpoints might be indicated. Also suggested by the studies is the need to look at levels of spectrin crosslinking in workers in the rayon industry in this country and in central and eastern Europe, and try to determine what levels correlate with measures of clinical neurotoxicity.

Atherosclerosis Study, Preliminary Results — Dr. Graham reported that in addition to neurotoxicity, workers exposed to CS_2 show an increased incidence of atherosclerosis. In the final study to be reported, mice receiving either a normal diet or one high in fat were exposed to CS_2. He said that the form of circulating cholesterol most convincingly related to development of atherosclerosis is low density lipoprotein or LDL, and it is thought that oxidation of LDL allows it to be taken up by macrophages leading to formation of foam cells, which are the initial lesions in development of the atherosclerotic plaque. Dr. Graham said there is some evidence that covalent crosslinking of the apolipoprotein B component of LDL may precede uptake of LDL by macrophages. His laboratory recently reported that LDL exposed to CS_2 will also result in apo B crosslinking. Dr. Graham said that if this is the mechanism through which CS_2 accelerates atherosclerosis, implications would be profound. First, any toxicant that is an oxidant or could be metabolized to an oxidant could accelerate the process, or secondly, any toxicant that is a protein crosslinking agent or is metabolized to a protein crosslinking agent could accelerate the rate of atherosclerosis.

Dr. Jim Lewis, Duke, described a study in progress employing a mouse model, the C57Bl6 mouse on a high fat diet. This mouse is susceptible to atherosclerosis and its macrophage properties are very well defined. The same doses were used as in the principal study and dose groups were taken out up to 20 weeks. These animals develop atherosclerosis right under the valve cusp in the aorta. The findings were that both the incidence and severity of the lesions were markedly increased in animals exposed to CS_2.

Acknowledgments and Comments — Dr. Sills thanked the Board for their advice and recommendations, noting that Dr. Klaassen had earlier suggested that atherosclerosis be an endpoint. He thanked the research team for an outstanding collaboration. Dr. Sills thanked Dr. Lucier, Dr. Bucher and Dr. Boorman for their support and vision in providing opportunities where the NTP extends its long-term studies to include mechanistic and biologically based research to aid in the risk characterization process.

Discussion — Dr. Goldsworthy inquired as to where the NTP would go from here with CS_2. Dr. Sills replied that the big issue would be evaluating low dose effects over a longer time period on the various endpoints and the cumulative effect. Dr. Klaassen asked whether the study had been designed as a precursor to a chronic carcinogenicity study or primarily to study neurotoxicity of a known neurotoxic agent. Dr. Sills said that before considering long-term studies, it was felt that biologic and mechanistic data be developed to try and understand the endpoints most critical to human health. Dr. Miller wondered if this sort of approach could be translated into requests for proposals for studying other chemicals. Dr. Lucier responded that a new series of R03 grants had been established which would allow us to get information of a mechanistic nature on various endpoints by providing extramural investigators with samples and tissues from NTP studies on specific chemicals.

These Summary Minutes (for the June 20-21, 1995, meeting) have been read and approved by the National Toxicology Program Technical Reports Review Subcommittee. They are certified by the Chair of that Subcommittee below.

Date: _____

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Arnold Brown, Ph.D.