

***ITER* Peer Review Meeting Summary on Carbon Disulfide**

May 17, 1999
Carbon Disulfide

An independent panel of expert scientists and risk assessors met in Ottawa, Ontario to review a hazard identification and dose-response assessment on carbon disulfide. Health Canada developed the assessment on carbon disulfide as part of the Priority Substances Program under the Canadian Environmental Protection Act. This meeting was conducted by Toxicology Excellence for Risk Assessment (*TERA*); a non-profit organization dedicated to the best use of toxicity data in risk assessment. Expert peer reviewers donated their time and talents to provide an independent review of the assessment. A comprehensive overall review of the materials was provided by the combined experience of all the reviewers.

After brief introductions, the meeting began with a discussion of conflict of interest. Each reviewer certified that he or she did not have a conflict (real or apparent) with the chemical under review or sponsor, or identified the potential for such conflicts. Possible conflicts were discussed with each reviewer to determine if measures were needed to manage a potential conflict (or appearance of conflict). Options may include excluding the reviewer from a particular discussion and consensus, or allowing the reviewer to participate in the discussion, but not be polled for consensus. Panel members each identified themselves, summarized their backgrounds, and noted any possible conflicts. The panel agreed to each participant's participation as documented in Attachment A.

These review meetings follow a standard format beginning with a close examination of the supporting documentation and important references by the panel in the several weeks prior to the meeting. At the meeting, after the conflict of interest discussion and decision by the panel is made, the authors of the assessment or documentation briefly present their work. For chemical assessment documents, the panel then systematically discusses the assessment, starting with a discussion of the qualitative weight of evidence and a determination of whether adequate data exist on which to base a risk value, followed by a discussion of the appropriate critical endpoint and studies. Next, the quantitative aspects of the assessment are discussed, including proposed cancer risk estimates and reference doses and concentrations, as appropriate.

Full discussion and participation are encouraged and agreement is reached by consensus. Consensus for the purpose of these meetings is defined as "an opinion held by all or most, or general agreement." The meeting was open to the public and an observer from the U.S. Occupational Safety and Health Administration was present.

Assessment for Carbon Disulfide

Sponsor: Health Canada

Presenters Ms. Bette Meek, Health Canada
Mr. Ron Newhook, Health Canada
Dr. Michael Walker, Health Canada
Chair: Dr. Michael Dourson, *TERA*

Review Panel:

Dr. Robert Bornschein, University of Cincinnati, Dept. of Environmental Health
Dr. John P. Christopher, California EPA, Dept. of Toxic Substances Control
Mr. Harvey J. Clewell III, K.S. Crump Group, ICF Kaiser International
Dr. Michael L. Dourson, Toxicology Excellence for Risk Assessment (*TERA*)
*Dr. Daniel J. Guth, The Boeing Company, Safety, Health & Radiation Protection
Dr. Mary Prince, National Institutes of Occupational Safety & Health (NIOSH)
Dr. William Valentine, Vanderbilt University Medical Center, Dept. of Pathology

*Provided written comments and did not participate in discussion or consensus decisions.

The meeting began with a review of the process, ground rules, and conflict of interest management plan. The panel agreed with the proposed plan for managing conflict of interest with the change to note that Dr. Daniel Guth was a principal author of U.S. EPA's Reference Concentration (RfC) currently on IRIS, and thus can be considered to have taken a prior public position. However, Dr. Guth no longer works for EPA. Dr. Guth did not attend the meeting; rather he submitted written comments for consideration by the panel and Health Canada. His comments were presented and considered during the discussion.

Ms. Bette Meek and Mr. Ron Newhook of Health Canada presented information on the carbon disulfide assessment and Health Canada's goals for the peer review. Dr. Michael Walker of Health Canada presented information and answered questions concerning the benchmark concentration modeling. The review panel discussed the assessment document, evaluating the hazard characterization and dose-response assessment. Below is a summary of their discussions and conclusions.

PRESENTATION

Ms. Meek briefly discussed the objectives for the review and development of the assessment document. She noted that the document being reviewed would be part of unpublished supporting documentation for the files. The final published Assessment Report will be shorter and contain only the information critical to the hazard characterization and dose-response analyses. The final published document will also contain an exposure characterization (not the subject of this review).

Mr. Newhook of Health Canada presented information on the carbon disulfide assessment. There is an extensive database on the effects of carbon disulfide in humans. Cardiovascular effects associated with exposure to carbon disulfide include increased mortality from heart disease and alterations in risk factors (e.g., blood pressure,

cholesterol). Limited data suggest increased morbidity. This is supported by animal studies. Human data also indicate that neurophysiological and behavioral effects are associated with exposure to carbon disulfide; these endpoints are also supported by animal studies. The weight of evidence is strongest for nervous system effects with consistent effects on peripheral nerve conduction velocity (NCV).

The proposed principal study was Johnson et al. (1983), in which a cohort of rayon staple workers were studied by the U.S. National Institute of Occupational Safety and Health (NIOSH). For this assessment, the individual data were obtained, and benchmark concentrations (BMCs) were calculated using the method of Crump (1995) for continuous data. The modeling was conducted using two definitions of background adverse response level (first and fifth percentiles). The BMCs (maximum likelihood estimate of response), and lower confidence limits (BMCLs) were calculated for benchmark response (BMR) levels of 0.01, 0.05, 0.1, and 0.2. BMCs were calculated for responses significantly related to exposure, including the motor nerve conduction velocity (MCV) of the peroneal nerve, sensory conduction velocity (SCV) of the sural nerve, diastolic blood pressure, and low-density lipoprotein (LDL)-cholesterol.

The proposed Tolerable Concentration (TC) was calculated using the lower bound on 5% BMR (the $BMCL_{05}$), using a 5% adverse response. The critical effect was decreased MCV of the peroneal nerve in the cohort of Johnson et al. (1983). The resulting $BMCL_{05}$ was 13 mg/m^3 . The $BMCL_{05}$ was multiplied by $8/24$ and by $5/7$ to adjust from the intermittent exposure under occupational conditions to continuous exposure. An uncertainty factor of 50 was used (10 for intraspecies variation and 5 to account for the lack of data on potential neurodevelopmental effects). The resulting TC is 0.06 mg/m^3 .

DISCUSSION

Hazard Characterization

Several reviewers noted that the document was quite well written. There were a few clarifications sought on the text, however. One reviewer asked why data on effects of carbon disulfide on hearing were not included. Health Canada replied that such data might have been screened out at an early stage, if exposure data were not available. The reviewer stated that carbon disulfide is one of the few clearly ototoxic chemicals known, and that a key epidemiological study of ototoxicity in Brazil, by Morata (1989), should be included. Carbon disulfide interacts with noise, and can cause hearing loss as a result of both central nerve and peripheral nerve damage. The reviewer agreed, however, that the effects on hearing occur at higher exposures than those on nerve conduction velocity and thus the reviewer did not think that the missing data would affect the assessment. The panel agreed, however, that this endpoint should be included in the hazard characterization for completeness.

One reviewer asked why the document concluded that an exposure-related effect in the Reinhardt et al. (1997a) study existed, when the study authors did not consider the observed differences to be exposure-related. Health Canada replied that this consideration

is a judgement call, and the opinion of the panel was solicited. Health Canada's conclusion was based on a consideration of the reasons that the study authors gave for discounting the differences, in light of a comparison of the Reinhardt results with the overall database. The study authors discounted the effect for four reasons:

1. The study authors believed that a decreased amplitude of the action potential should precede the decrease in nerve conduction velocity;
2. they believed that a prolonged distal motor latency should precede the decreased NCV;
3. no decrease in the SCV was observed; and
4. no statistically significant dose-response was seen for MCV using any exposure measure, although a statistically significant effect was seen in a binary comparison between the exposed and control groups.

With regard to the first three points, Health Canada noted that the same patterns were observed in the principal study of Johnson et al. (1983), and the evidence is clearer for an effect of carbon disulfide on MCV than on SCV. With regard to the fourth point, Health Canada determined that the dose-response in the exposed group should not be weighted over a comparison between the exposed and control group. One reviewer disagreed with the expectations of Reinhardt and colleagues with regard to the order of pathophysiological effects, and stated that the effects (and absence of effects) observed in the Reinhardt study are consistent with what is known about carbon disulfide. The mechanism of action of carbon disulfide is discussed below in the section on the choice of critical effect. Another reviewer noted that the exposure in the Reinhardt study was near the threshold, and that it is reasonable that the results would be equivocal. The panel concurred with the judgment of Health Canada and suggested that the document authors make more explicit the reasons for considering the changes in the Reinhardt study to be compound-related.

Choice of Critical Effect

The proposed critical effect was decreased MCV in the peroneal nerve observed in the NIOSH cohort (Johnson et al., 1983). The panel also discussed the cardiovascular endpoints and the respective benchmark concentrations to reach a conclusion regarding the selection of critical effect.

To expand the panel's understanding of the significance of the neurological effects, one reviewer presented additional information on the mechanism and progression of the neuronal effects of carbon disulfide. For carbon disulfide, decreased nerve conduction velocity is an important endpoint, and is indicative of the loss of axons. In experimental animals the distal portions of the largest and longest myelinated axons (which are the most rapidly conducting axons) are affected first. Structural changes proceed through the development of large axonal swellings composed of disorganized masses of neurofilaments proximal to nodes of Ranvier, followed by axonal atrophy and Wallerian-like degeneration proximal and distal to the swellings, respectively. The animal data are consistent with reports on humans that found the motor conduction velocity obtained

from the peroneal nerve of the leg to be decreased before the conduction velocities obtained from the sural nerve and ulnar nerve. (The sural and ulnar nerves contain axons that are smaller in diameter and shorter in length, respectively, compared to the peroneal nerve.) Interspecies susceptibilities can also be interpreted based upon the length and diameter of axons, with rats being more susceptible than mice and humans expected to be more susceptible than rats. Although neurobehavioral changes have been detected prior to axonal structural changes at the light microscopic level, the changes observed (i.e., ataxia, leg splay, decreased hind limb grip strength, etc.), are all consistent with dysfunction of the distal segments of motor neurons.

This reviewer further noted that NCV is a relatively crude indicator of nerve degeneration for carbon disulfide, because function is not impaired until axonal degeneration has actually occurred, in contrast to agents that produce demyelination or have a direct effect on conduction. In other words, carbon disulfide-mediated decreases in NCV indicate axonal degeneration in peripheral nerves. It is not possible, however, to identify a quantitative comparison between a given decrease in NCV and an expected degree of loss of function. An additional concern is that, although an effect in the peripheral nervous system (PNS) is measured, because carbon disulfide produces a central-peripheral distal axonopathy, this endpoint may also indicate that the central nervous system (CNS) has also been affected. The reviewer noted that there is limited capacity for regeneration in the PNS, and that the CNS has even less capacity for regeneration. The panel noted that, while the endpoint of decreased NCV *by itself* may not be adverse, it is indicative of, and a precursor of, other changes that clearly are adverse. Based on the mechanistic considerations, the panel recommended that the revised document refer to this endpoint as "preclinical," rather than "subclinical," defining the critical effect as a statistically significant, compound-related decrease in NCV. The panel recommended that Health Canada enhance the explanation of the critical effect and its significance in their documentation, on the basis of the presented information.

The reviewer further clarified that, on a molecular level, the key event for the development of axonal swellings appears to be covalent cross-linking of neurofilament subunits. The function of neurofilaments is unclear, but they may play a role in maintaining the volume of the axon. Neurofilaments are synthesized in the cell body and travel down the axon to the terminus, at the rate of approximately 1-2 mm/day. During this transit, the neurofilament subunits maintain a close spatial proximity and are vulnerable to cumulative covalent cross-linking by carbon disulfide. Although some level of cross-linking is non-adverse to the organism, as evidenced by the detection of neurofilament protein cross-linking detected in the absence of axonal structural changes, a sufficiently high level of cross-linking results in the development of axonal swellings. Transit of neurofilaments appears to be interrupted at constrictions of the axon at nodes of Ranvier, possibly due to the formation of "logjams" of neurofilaments. Whether neurofilaments are the target responsible for the subsequent axonal degeneration has not been determined, but a role for covalent protein modification and cross-linking in axonal degeneration has been supported by investigations on 2,5-hexanedione, a compound that produces an axonopathy identical to carbon disulfide. The reviewer noted that the term "dying back" used by Reinhardt et al. (1997a) is not an appropriate characterization.

Instead, the entire distal segment of the axon degenerates simultaneously, similar to the Wallerian degeneration that occurs following transection of the axon.

The panel discussed whether comparisons to the general population would be useful, particularly in determining what "normal" values are. It was noted, however, that the control values in the Johnson et al. (1983) study were approximately 10% lower than reference values. Because of the potential for inter-laboratory differences NCV measurement methods, and because the Johnson study was well controlled, the panel concluded that it was more important to compare the data with the concurrent controls than with general population reference values.

The panel also discussed the appropriateness of cardiovascular endpoints as the critical effect. A reviewer noted some problems with labeling the cardiovascular endpoint as the critical effect:

1. the mechanism of the cardiovascular endpoint is unclear, but is likely to be nonspecific;
2. mediating factors are likely to be important for the cardiovascular endpoints; and,
3. there is a high background for these endpoints, so the dose-response curve is more shallow and there is more variability.

This same reviewer believes that carbon disulfide does cause cardiac effects, although at higher exposures than for the NCV endpoint. The reviewer continued that it is rare to observe cardiac effects in cohort studies, so the observations of any statistically significant difference between exposed and control groups is likely a biologically significant chemical-related effect. Another reviewer noted that the mode of action for cardiac effects is likely to be different from that for neural effects (the former may be due to effects on lipid metabolism), and so the dose-response curves for the two endpoints may differ. A different reviewer noted the importance of specifying which cardiovascular endpoint is affected. The reviewers suggested that the document was confusing regarding the implications of a shallow dose-response curve on the BMC; and the panel recommended that the statement regarding the shallow dose-response curve be removed.

At the request of the panel, Dr. Michael Walker of Health Canada presented results of the BMC modeling for the cardiovascular risk factor endpoints of diastolic blood pressure and low density lipoprotein (LDL) cholesterol. For a 5% background adverse response level, the $BMCL_{05}$ values for these two endpoints were 7.6 and 7.2 ppm, respectively. (For comparison, the $BMCL_{05}$ for peroneal MCV, the most sensitive neural endpoint, using a 5% background adverse level, was 4.1 ppm.) This modeling was conducted using the same data set as the modeling for the Johnson et al. (1983) data. [The cardiovascular results were initially published as Egeland et al. (1992).] For both endpoints, the individual data were obtained, and adjustment for confounders was conducted using multiple regression analysis (see the section on BMC for more information). It was noted, however, that the higher background levels for cardiac effects in the general population would result in a lower BMC. One reviewer estimated the background rate of adverse response (in the general population) at 20-25% for this endpoint, but Health Canada had

not calculated the BMCs for this endpoint using that background adverse response level. Another reviewer estimated that the BMCs with a background adverse response level of 20-25% would be expected to be comparable to, or slightly higher than, the BMC for the proposed critical effect.

Overall, the panel considered the BMC for the neural effect to be protective for cardiovascular endpoints, even if a higher background level of adversity were used for modeling the cardiovascular data. Health Canada also emphasized that the criteria for causality were not adequately met for the cardiovascular endpoint, and thus this endpoint was not recommended as the critical effect.

The panel recommended that the quantitative results of the BMC modeling for the coronary risk endpoints should be presented in the documentation, along with the neural endpoints, to help support the choice of critical effect. The panel agreed that the neural endpoint is preferred, based on the weight of evidence for causality. The panel recommended that the text regarding the higher plausibility and specificity of the NCV endpoint over the cardiovascular endpoint, and the importance of identifying the specific cardiovascular endpoint, be enhanced.

The panel addressed how the data on developmental neurotoxicity should be taken into account in the hazard characterization. Health Canada asked whether the potential developmental neurotoxic effects associated with carbon disulfide were adequately addressed. The panel agreed that the Tabacova studies (Hincova and Tabacova, 1978; Tabacova et al., 1978, 1981, 1983) have methodological problems and inconsistencies that raise concerns about the reliability of the results. (These studies reported neurobehavioral effects at concentrations as low as 0.03 mg/m³, as well as malformations at this concentration in the second generation of a two-generation study.) Problems with these studies include inconsistencies in the identification of exposure levels and numbers of animals per group, a gap of more than two orders of magnitude between the low and mid concentrations, and lack of reporting on maternal toxicity. A reviewer noted that, even though the study is inconsistent with other studies in observing effects at much lower levels, it still might be valid to conclude that effects occur at lower levels in a multigeneration study than in a standard developmental toxicity study. Another reviewer noted that the magnitude of the shift in sensitivity between the F1 and F2 generations was almost without precedent. The general lack of developmental neurotoxicity studies was noted, and a reviewer asked that the document more clearly note this lack. Another reviewer stated that there is a Chinese epidemiology study on effects in the offspring of Chinese rayon workers. This reviewer will check on whether the study has been published and is available. The panel concluded that the document adequately addressed the endpoint, but should note more clearly the absence of a good multigeneration study, the absence of developmental neurotoxicity studies, and the importance and relevance of these data gaps.

The panel concurred with Health Canada's choice of decreased motor nerve conduction velocity of the peroneal nerve as the critical effect.

Mode of Action

The panel complimented the authors on the mode of action documentation. One reviewer agreed that the contribution of the carbon disulfide metabolite carbonyl sulfide (produced by mixed function oxidase metabolism) cannot be evaluated, but stated that the weight of evidence regarding cross-links resulting from carbon disulfide directly and those produced by its metabolite are rather different. Cross-links formed directly from carbon disulfide have been observed. By contrast, although metabolites resulting from carbonyl sulfide have been identified, the production of cross-links via this pathway has been hypothesized, but not shown. The protein cross-linking ultimately can result in axonal swelling and degeneration. Another reviewer noted that, since carbonyl sulfide is produced by mixed function oxidases, allometric considerations would mean that the contribution in humans would be expected to be lower than that in animals. The panel suggested that the text, and possibly the figure, for the mode of action discussion note the difference in the weights of evidence for the direct effect of carbon disulfide and of its metabolite. The panel also recommended that the paragraph regarding an alternative mechanistic hypothesis (i.e., oxidative desulphuration of carbon disulfide by microsomal enzymes, resulting in the production of reactive sulphur) be deleted, as too hypothetical. The panel agreed that the document appropriately drew comparisons to related chemicals.

Principal Study

The proposed principal study was Johnson et al. (1983), using the entirety of the individual database for this NIOSH cohort. One reviewer questioned whether the Vanhoorne et al. (1995) study had sufficient concentration-response data, and should be modeled. Health Canada replied that the exposures were well characterized in that study, and that the range of exposure was similar to that of other studies, but that the initial (1991) report of that cohort reported very high exposures. The revised document will clarify and slightly expand this point. Health Canada also noted that M. Vanhoorne was one of the earlier reviewers of the assessment, and did not disagree with the characterization of exposures in the Vanhoorne et al. (1995) study as having been relatively high. A reviewer also noted that the Vanhoorne study is the same cohort as the De Fruyt et al. (1998) study. The reviewer expressed concern about the possibility of selection bias in that study, given that 46% of the exposed group, but 100% of the controls, participated in the study.

The panel agreed with the selection of the Johnson et al. (1983) for the principal study.

Benchmark Concentration

Health Canada proposed to use a $BMCL_{05}$ for a 5% background adverse effect level, as the basis for the tolerable concentration. The most sensitive endpoint was peroneal nerve MCV, and the resulting value was 13 mg/m^3 . The panel discussed many issues, including the appropriate choice of benchmark response and the background adverse response level, use of the hybrid continuous model versus earlier versions of continuous models, whether selection of the BMR should take into account the irreversibility of the endpoint,

whether the BMC should be calculated using excess risk, the difference between the current assessment and Price et al. (1996), and whether current or cumulative exposure is a better predictor of toxicity.

There was considerable discussion regarding the appropriate choice of the benchmark response (BMR) and the background adverse response level (p_0). Health Canada noted that several different options were presented because this is not an easy choice. One reviewer recommended that the benchmark response (BMR) be defined based on a biological definition, rather than using a statistical basis. Another reviewer noted that this is a point of controversy in the benchmark modeling community. This reviewer argued for a statistical basis for the BMR, stating that it would be difficult to define a percent decrease in NCV that is adverse, and that statistical definitions of "normal" are used even in clinical settings. One reviewer noted that the definition of "abnormal" is based on statistical grounds (i.e., deviation from average), while the definition of adversity is based on judgment, and, on an individual basis, adversity is based on the entire clinical picture. The panel agreed that a statistical definition of abnormal is appropriate for this approach.

One reviewer noted that the modeling approach used for the continuous data was based on the approach of Crump (1995). (This method is sometimes called the "hybrid continuous" model, because it models continuous data, but expresses the results in terms of probability of response.) The reviewer noted that Dr. Crump prefers this method over the earlier continuous models (ICF Kaiser, 1990a, 1990b), and does not consider the theoretical basis for the earlier continuous models to be appropriate, due to the arbitrary nature of the definition of effect level. The reviewer noted that researchers in the field have recommended a background adverse response level of 5%, based on analogy to the clinical environment, where 5% of the population on either tail is considered "abnormal." The reviewer was pleased with the presentation in the document of the results for different modeling approaches, and the comparison to the NOAEL. This reviewer considered it plausible to use BMRs of either 5% or 10%, and reasonable to use a BMR of 5%. Health Canada noted that the reason for the BMR of 5% is based on analogy to Health Canada's use of the TC_{05} (Tumourigenic Concentration, 5%) for cancer. One reviewer was confused by the description of the modeling approach as "specifying a cutoff within the unexposed population that separates continuous responses into normal and abnormal categories." It was suggested that the text clarify that the model does this, and the data themselves are not quantalized.

One reviewer asked whether the choice of the BMR should be affected by the irreversibility of the endpoint. Another reviewer responded that there is some disagreement among researchers in the field, but that neither this reviewer, nor Dr. Crump, believe severity should influence the choice of BMR, since the aim is to identify the "true" NOAEL. Instead, the reviewer believes that severity of endpoint should affect the choice of uncertainty factors.

Reviewers had additional suggestions regarding the BMC calculations and associated text. The panel recommended that the figure of the benchmark modeling results be modified to show the upper and lower 95% confidence limits on the controls, to show

where the BMC falls on the graph, and that the text identifies the confidence limits lines. One reviewer suggested that the BMCs also be calculated using excess risk, for comparison with the calculations using additional risk.

The full panel agreed with the suggestion that excess risk be presented also recommended that the description of the line on the figure be enhanced to explain why the line is not a visual best fit of the (adjusted) data, although the underlying model is the best fit. Another reviewer requested that the explanation of how the correction is done be improved, and that the explanation be inserted after the presentation of the first equation.

The panel discussed whether current or cumulative exposure is a better predictor of toxicity. Health Canada noted that the modeling was done using both alternatives (current or cumulative exposure) as the exposure measure. Current exposure was a better predictor of response, but similar BMCs were obtained using either approach. Health Canada also noted that the measurement of cumulative exposure is much cruder than that for current exposure. They pointed out that the average exposure duration in the Johnson study was 12 years.

One reviewer requested that the modeling results using cumulative exposure be presented for comparison. With regard to mechanistic considerations of whether the effect would be cumulative, a reviewer reported that the extent of the cumulative nature of the effect within a given axon is limited by the lifespan of the neurofilaments as they pass down the axon from the cell body to the terminus. If exposure was long enough for the neurofilaments in a given axon to traverse the entire length of the axon, and the axon survived, exposure for a longer duration will not result in degeneration of that axon. The reviewer also noted that certain levels of neurofilament cross-linking are not adverse. Another reviewer proposed a bounding estimate of 3 years for the lifespan of neurofilaments (based upon an axonal length of 1 meter and transport rate of 1 mm/day). The reviewer did note, however, that at exposures where axons are affected, there could be a cumulative effect within a nerve due to the limited repair capacity. In other words, current exposure is indicative of whether an individual axon would be affected, but cumulative exposure (*if* exposure were above the threshold for effects) is an indicator for how many axons in a nerve would be affected. Based on these considerations, the panel considered current exposure to be the more scientifically valid measure of exposure, on mechanistic grounds, but requested that the results using cumulative exposure also be presented.

The panel agreed unanimously with Health Canada's choice to use the lower bound on the BMR of 5% (i.e., the $BMCL_{05}$), and a background response incidence of 5%, noting that these choices were consistent with Health Canada's approach for other chemicals.

Duration Adjustment and Dosimetry

Health Canada suggested the use of factors of 8/24 and 5/7 to adjust for discontinuous exposure. One reviewer asked whether an occupational minute volume is a more appropriate adjustment, by analogy to U.S. EPA's RfC methods. Another reviewer

responded that the minute volume is appropriate for particles, which cumulatively deposit. By contrast, gases such as carbon disulfide rapidly reach steady state, so the minute volume is not important. The panel concurred with Health Canada's suggested factors.

Uncertainty Factors

Health Canada proposed a composite uncertainty factor of 50. This was based on a factor of 10 for intraspecies variability, in the absence of sufficient data to use a data-derived factor, and a factor of 5 to account for potential neurodevelopmental effects. No uncertainty factor for the use of less than lifetime exposure was considered necessary, in light of the long exposure (mean 12.1 years) in the cohort, and because current exposure was a better predictor of an effect than cumulative exposure was.

One reviewer questioned whether the data on mode of action (binding to neurofilaments and cross-linking) is consistent with the statement that insufficient mechanistic data are available to use a data-derived uncertainty factor for intraspecies kinetics. Health Canada replied that their understanding of the data is that both carbon disulfide and its metabolite carbonyl sulfide can contribute to the cross-linking. Thus, metabolism would contribute to kinetic variability. Another reviewer noted that there are other aspects of kinetic variability beyond metabolism, and stated that the observed cross-linking is probably due to direct action of carbon disulfide, rather than a metabolite, but the data are not sufficient to reduce the toxicokinetic portion of the uncertainty factor. The absence of data on metabolism in humans was also noted. Additional reasons for including a full uncertainty factor for intraspecies variability were noted. Health Canada observed that there are potential sensitive subpopulations that would not have been included in the occupational cohort, including the elderly (because of the age-related decrease in NCV, this group would have less reserve), and diabetics (who are prone to polyneuropathy). One reviewer suggested that children are probably at lower risk than adults are, because their neurons are shorter. The panel recommended that the discussion of this uncertainty factor should be expanded to include the above points, as well as noting the crudeness of the critical effect (because it indicates that significant damage has already occurred), and the insufficiency of the data for use of a data-derived uncertainty factor.

Regarding the uncertainty factor for potential neurodevelopmental effects, the panel agreed with Health Canada on the significance of deficiencies in the database. Health Canada noted that they had done a direct comparison of concentrations associated with neurobehavioral effects in young animals that had been exposed *in utero* and in adults. The panel recommended that this analysis be presented (perhaps in tabular form, or comparing particular studies), to support the statement that developing offspring may be more sensitive to the neurological effects of carbon disulfide. In light of these considerations, the panel considered the lack of a multigeneration study that evaluated neurotoxicity to be a meaningful data gap.

The panel agreed that the uncertainty factor for database insufficiencies should be a value between 1 and 10, and that Health Canada's choice of 5, based on their methodology, was reasonable.

The panel also agreed that it was not necessary to account for the less than lifetime exposure in the choice of uncertainty factor, based on the mechanistic considerations described above.

As a result of the panel's agreement with Health Canada's choice of critical study, BMD, duration adjustment and dosimetry, and uncertainty factors, the panel unanimously agreed with the calculation of the TC as proposed (0.06 mg/m³).

The panel had some general suggestions on the presentation of the material in the assessment document, which were communicated verbally to Health Canada.

Oral Tolerable Intake and Carcinogenicity

Health Canada did not develop an oral tolerable intake, based on the limitations of the available data. It was also noted that this is consistent with the Health Canada approach, which considers a contribution to total exposure of less than 10% to be insignificant. One reviewer also noted the low water solubility of carbon disulfide.

The panel concurred with Health Canada's determination that it is not appropriate to derive a tolerable intake, but recommended that the document note that anticipated lower oral absorption (as compared to inhalation absorption) would mean that any route-to-route extrapolation would likely be conservative.

The panel also agreed that the discussion in the document regarding carcinogenic potential was appropriate.

PANEL RECOMMENDATIONS

The panel concurred with Health Canada's conclusions and the tolerable concentration. Below is a summary of the panel's specific recommendations for revision to the assessment.

Hazard characterization

1. A reviewer volunteered to provide Health Canada with the Morata (1989) epidemiology study on ototoxicity of carbon disulfide, and Health Canada agreed to modify the document to note this as an effect, and the potential for carbon disulfide to interact with noise.
2. The panel recommended that Health Canada expand the explanation for its evaluation of the Reinhardt et al. (1997a) study, and why its conclusion differed from that of the study authors, including noting similarities to the results of the Johnson et al. (1983) study.

3. The panel recommended that Health Canada expand the text regarding the adversity of the critical effect (decreased nerve conduction velocity) to note the following points:
 - The effect is *preclinical*, rather than *subclinical*
 - The endpoint is relatively crude, in that damage to axons has already occurred by the time decreased NCV can be measured
 - Although the endpoint measured is in the peripheral nervous system, it is possible that central nervous system effects are occurring at the same levels.
 - The endpoint has limited reversibility in the peripheral nervous system
 - There is a difference between abnormality (a statistical result based on comparison with the population as a whole), and adversity (a judgement based on the overall data available).
 - The critical effect is defined as a statistically significant, compound-related decrease in the nerve conduction velocity.
4. The panel recommended that Health Canada modify the text to better explain the choice of the nervous system endpoint over the cardiovascular endpoints, including:
 - Presentation of the BMC results for the most sensitive coronary risk factor endpoints;
 - More clear presentation of the argument that the nervous system data meet the criteria for causality and plausibility better than the cardiovascular data do; and
 - Removing the statement about the significance of the shallower dose-response curve for these endpoints.
5. A reviewer will attempt to obtain a recent Chinese study evaluating developmental neurotoxicity of carbon disulfide, if it has been published.

Mode of Action

1. The panel recommended that appropriate figure in the document and corresponding text be clarified to note that the production of protein cross-links by carbon disulfide metabolites has only been proposed, while the cross-links produced by direct reaction with carbon disulfide have been measured.
2. The panel recommended that text be added providing additional information on the mechanism of the nervous system effects of carbon disulfide, including a discussion of the limitations to the cumulative nature of the effect.
3. The panel recommended that the paragraph suggesting reactive sulphur (from oxidative desulphuration of carbon disulfide) binding to thiol groups of enzymes be deleted, as too speculative.

Dose-Response

1. The panel recommended that the plot of the benchmark concentration data should be modified to show the upper and lower 95% bounds on the control population response, and possibly should include a line indicating where the chosen BMC lies.
2. The panel recommended that Health Canada should include a calculation of the BMC using excess risk.
3. The panel recommended that the text should be modified to clarify that the continuous data were not converted to quantal data. Instead, a modeling approach was used that models the continuous data, but expresses the resulting risk as the probability of response.
4. The panel recommended that results of the modeling using cumulative exposure should be presented, although the panel agreed that the TC should be based on the current exposure.
5. The panel recommended that the rationale for choice of a factor of 10 for intraspecies variation be expanded to include explanations of several points. As noted in the document, the data are insufficient to replace a default value with a data-derived value for toxicokinetics. In addition:
 - there are potential sensitive populations that may not have been adequately covered in the occupational epidemiology studies (the elderly, and diabetics);
 - the metabolism of carbon disulfide is not fully known, particularly in humans; and
 - the endpoint of nerve conduction velocity is a rather crude one, in that axonal damage has occurred by the time the decrease is measurable.
6. The panel recommended that the text regarding the uncertainty factor for database insufficiency be modified to include the following points.
 - Tie the text better to the text in Section 12.3 regarding uncertainties in the effects of carbon disulfide on neurobehavioral development.
 - Note the uncertainty regarding potential effects on the central nervous system.
 - Add text and possibly a table, explaining the analysis in which Health Canada compared effect levels in adult and young animals for neurobehavioral endpoints.
 - Note the gap of a well-conducted multigeneration study that evaluated neurobehavioral endpoints.

Tolerable Intake Calculation

1. The panel recommended that Health Canada add text noting the low absorption via the oral route, so that readers would be aware that a direct route-to-route extrapolation from the inhalation data (without addressing absorption) would be conservative (health protective).

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Attachment A
Managing Potential Conflicts of Interest
***ITER* Peer Review Meeting**
May 17, 1999
(accepted by panel)

ITER peer reviewers donate their time and talents to this effort. They are selected based upon their expertise and qualifications and are employed by many types of organizations. *TERA* strives to create a balance of expertise and affiliations for each meeting. However, individual peer reviewers are representing their own expertise and views, not those of their employer. The *TERA* Board of Trustees approves *ITER* peer reviewers for inclusion in this program. A complete list of potential reviewers and more information on the *ITER* peer review program are available at <http://www/tera/org/peer>. Additional, *ad hoc* reviewers are selected to participate for their special expertise that may be needed for a particular chemical or discussion.

TERA requested that each peer reviewer identify potential conflicts of interest related to the review of the health risk assessment of carbon disulfide, and/or the sponsor, Health Canada. Each reviewer has signed a statement indicating that he or she does not have a conflict of interest concerning this assessment.

The following statements were considered by the panel and agreed upon at the meeting.

Robert Bornschein – Dr. Bornschein is a professor at the University of Cincinnati, Department of Environmental Health. He has no conflicts and will participate fully in all discussions and polling for consensus.

John Christopher - Dr. Christopher is a Toxicologist with the Department of Toxic Substances Control of the California Environmental Protection Agency (Cal EPA). Cal EPA regulates various aspects of production, use, sale or disposal of many chemicals, including that under discussion. However, Dr. Christopher does not have a specific conflict of interest with this chemical and will participate fully in the discussion. Dr. Christopher requested inclusion of the following note: "Dr. John Christopher performs

scientific peer review for *TERA* as a private individual. His employer, the California Department of Toxic Substances Control, is not bound in any way by the opinions he expresses or by consensus agreements to which he chooses to be a party."

Harvey Clewell III – Mr. Clewell is with the K.S. Crump division of ICF-Kaiser International. He has no conflicts and will participate fully in the discussion and polling for consensus.

Michael Dourson – Dr. Dourson is Director of Toxicology Excellence for Risk Assessment (*TERA*). Dr. Dourson will serve as panel chair. He has no conflicts and will participate fully in all discussions and polling for consensus.

Daniel Guth - Dr. Guth is a Toxicologist with the Boeing Company. He has no conflicts. Dr. Guth cannot attend the meeting but has provided written comments that will be considered by the other panel members. . The panel noted that Dr. Guth was a principal author of U.S. EPA's Reference Concentration (RfC) currently on IRIS, and thus can be considered to have taken a prior public position. However, Dr. Guth no longer works for EPA. His comments were presented and considered by the panel during the discussion.

Mary Prince – Dr. Prince is an Epidemiologist with the National Institute for Occupational Safety and Health of the U.S. Centers for Disease Control. She has no conflicts and will participate fully in the discussion and polling for consensus.

William Valentine – Dr. Valentine is a neuropathologist in the Department of Pathology at Vanderbilt University Medical Center. Dr. Valentine reviewed an earlier draft of the Supporting Documentation for Health Canada; he has not reviewed the Hazard Characterization and Exposure-Response Analyses, which are the focus of this review. This previous involvement does not constitute a conflict of interest and Dr. Valentine will participate fully in the discussion and polling for consensus.