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EXPERT REVIEW OF PHARMACOKINETIC DATA: FORMALDEHYDE

Final Evaluation Report

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TABLE OF CONTENTS

continued-

Table of Contents - continued

LIST OF FIGURES

1.0 INTRODUCTION

This report provides a summary of the discussions and conclusions from the review meeting conducted on December 2 and 3, 1985 under Work Assignment (WA) No. 07, "Expert Review of Pharmacokinetic Data: Formaldehyde" of Contract No. 68-02-4228. The preliminary draft of this report was produced on-site by the meeting participants to meet the U.S. Environmental Protection Agency (EPA) schedule. This final report has undergone final technical review by all review team members.

2.0 BACKGROUND

2.1 Technical

The Environmental Health Committee of EPA's Science Advisory Board (SAB) reviewed the draft report "Preliminary Assessment of Health Risk to Garment Workers and Certain Home Residents from Exposure to Formaldehyde," dated May 31, 1985, prepared by the Office of Toxic Substances (OTS). One outcome of the review is the committee's finding that the formaldehyde (CH_2O) assessment "will not be scientifically adequate without an analysis of the pharmacokinetic information and appropriate modification (of the assessment) based on this analysis." The committee is concerned with both the broad question: "How can one realistically begin to incorporate relevant kinetic information into quantitative cancer risk assessments?" and the specific issue of whether the pharmacokinetic data published in a study by Casanova-Schmitz et al. (1984) can be used in EPA's assessment of CH_2O.

The OTS agrees that appropriate pharmacokinetic data should be presented and considered in the risk assessment; however, in its draft risk assessment of CH_2O, OTS agreed with an analysis performed by Cohn et al. (1985) that the use of the Casanova-Schmitz data would be premature. OTS found that the Casanova-Schmitz data do not support a modification of the risk estimated for CH_2O; and, thus, the OTS did not utilize the data either qualitatively or quantitatively in its risk assessment. However, to address the concern of the Environmental Health Committee, OTS desired an independent, objective, expert analysis of the issue so that it may reconsider the appropriateness of using the data in its risk assessment.

Consequently, OTS authorized assembly of a team of expert scientists to conduct an evaluation of the Casanova-Schmitz study and any relevant underlying data developed in the study and to prepare an expert report that answers the question whether the study provides data that should/could be used in the CH_2O risk assessment.

To accomplish this effort each member of the team of expert scientists was initially provided the documents listed in Appendix 1. Additional references, listed in Appendix 2 were provided subsequently to each expert. The experts were asked to evaluate independently the documents and determine the extent to which the pharmacokinetic data are appropriate for use in the CH_2O cancer risk assessment. In conducting their evaluation, the experts were asked to consider specifically each of the questions presented in Appendix 3 insofar as their expertise allows. They were also asked to identify any underlying data (e.g., lab books, data tables, etc.) desired from the Chemical Industry Institute of Toxicology (CIIT) which would be of assistance in addressing the specific questions. The list of information/clarification requested from CIIT by the experts is presented in Appendix 4.

2.2 Administrative

Upon receipt of the WA from EPA, efforts were initiated to assemble a seven-member team of experts in metabolism, DNA adducts and statistics. The review

team members are identified on the List of Participants at Appendix 5. Dr. Lemone Yielding agreed to serve as the Team Leader and Review Meeting Chairperson. As such, Dr. Yielding served as the principal author/editor of the technical portions of this report. Other individuals representing EPA and ICAIR, Life Systems, Inc., at the review meeting are also listed in Appendix 5.

The review meeting was conducted on December 2 and 3, 1985 at the Sheraton University Center, Durham, NC. The Agenda prepared for the meeting is provided at Appendix 6. Although the Agenda was prepared for a three-day meeting, all Agenda items were completed at an accelerated pace and the meeting only required two days. On-site clerical facilities were provided during the meeting to prepare the draft report.

3.0 DISCUSSION

The following provides a summary of the discussions and evaluations conducted at the review meeting regarding the lists of questions/issues presented in Appendix 3 and Appendix 4.

Prior to the review meeting, written answers, statements and/or opinions were prepared by the expert reviewers on each of the questions/issues presented in Appendix 3. These responses were provided to EPA immediately following the meeting. They are not provided in this report because they have been superceded by the consensus opinions developed at the meeting and provided below.

The representatives from CIIT, listed in Appendix 5, participated in informal discussion with the expert reviewers. This discussion was in response to the expert's requests for information/clarification listed in Appendix 4 and included an expanded question and answer session during which further details were provided on CIIT's prior, ongoing and planned studies of CH_2O.

3.1 Distinguishing between Metabolically Incorporated and Crosslinked CH_2O

Questions/issues Nos. 1 and 3 of Appendix 3 were addressed together and are discussed below.

3.1.1 Metabolic Incorporations Versus Adduct Formation or Crosslinked CH_2O

The experimental evidence for metabolic incorporation of crosslinked CH_2O is largely based upon the relative incorporation of ^3H or ^{14}C-CH_2O into DNA of respiratory epithelium. The evidence presented in the written documentation was suggestive but not definitive in regard to this assumption. Additional evidence, presented at the interview with the CIIT staff, indicated that the $^3H/^{14}C$ ratio of the purine deoxyribonucleosides isolated from aqueous DNA by high performance liquid chromatography (HPLC) was in accordance with metabolic incorporation and furthermore, no shifts in pattern suggesting adduct formation were observed. It was not clear whether or not these types of experiments had been performed at all CH_2O levels. Interaction of CH_2O with tetrahydrofolic acid (THFA) could yield N5-10 methylene THFA directly, or following oxidation to formate could yield N-10 formyl THFA. The occurrence of these reactions, the equilibration of the various THFA derivatives and the reaction of the THFA derivatives with various one carbon acceptors could markedly influence the DNA $^3H/^{14}C$ ratio in metabolically-incorporated CH_2O. The pool sizes of the nonradioactive acceptors and their metabolic intermediates could also markedly influence the DNA $^3H/^{14}C$ ratio due to metabolic CH_2O. The relative reaction rates and pool sizes are likely to vary under different conditions. Thus, the interpretation of the $^3H/^{14}C$ ratio is very complex. This complexity necessitates the isolation of the DNA bases (or deoxyribonucleosides) and amino acids and the comparison of the $^3H/^{14}C$ profiles with authentic standards under conditions which separate any adducts

from the standards. Since it is not clear that this was performed at all CH_2O doses, doubt remains as to the assumption which formed the basis of distinguishing metabolically incorporated and crosslinked (or adducted) CH_2O.

3.1.2 Crosslinked CH_2O Located Exclusively in the Interface (IF) DNA

It is stated by Casanova-Schmitz et al. that all the crosslinked CH_2O was present in the IF rather than the aqueous (AQ) DNA. This assertion was not borne out by the written or oral documentation. Several control experiments which would have solidified the experimental basis for this assertion were not performed. The relative efficiency of extraction of the DNA from respiratory epithelium under conditions of CH_2O dosing (at various levels) should have been determined. That is, what is the recovery of the total DNA from the respiratory epithelium by the phenol procedure? Furthermore, the specific distribution of crosslinked DNA-protein in the IF-DNA fraction should have been affirmed. There is no doubt that at least some of the crosslinked CH_2O (DNA-protein) is found in the IF-DNA fraction. Whether all of this crosslinked fraction was in fact initially extracted from the tissue or whether some might be found in the AQ-DNA are questions which have not have been satisfactorily addressed. These experiments could have been performed by adding radioactively-labeled DNA (or crosslinked DNA-protein) to a tissue homogenate and following its distribution into the various fractions of the phenol procedure, i.e., a standard recovery experiment. Consequently, we believe that sufficient detail for characterization of the IF-DNA fraction has not been provided. This detail is absolutely necessary in order to validate the use of IF-DNA as a measure of crosslinked CH_2O, and therefore as a biological dosimeter.

3.2 Experimental Methodology Limitations

The inhalation methodology used to administer CH_2O was appropriate and the control and monitoring of CH_2O concentration as well as the isotopic composition of the gas mixtures employed were adequate. Infrared monitoring was done continuously during exposure and the instruments and methods crosschecked with other instruments and other methods. Variations in CH_2O concentrations were small compared to other variables and are not likely to have much impact on the experimental results. These factors are not considered to represent an important source of error in the experimental protocol. In considering the interpretation of the data in relation to human risk assessment, it should be borne in mind that inhalation by rats is restricted to the nose. In humans, inhalation can be expected to occur through both the nose and the mouth. The implications of this distinction with respect to human risk are unclear but always represent a source of uncertainty when rodents are employed as human surrogates in inhalation studies.

The DNA extraction procedure employed requires further validation since it is of central importance in distinguishing between the aqueous and interfacial DNA fractions on which the measurements of metabolic incorporation and crosslinking depend. There is no indication of the percentage of the total DNA recovered by this procedure, and it is likely that the amount of DNA associated with the interfacial fraction will vary to some extent with the extraction conditions employed (e.g. ionic strength, temperature, etc.). A standard recovery experi-

ment as outlined in 3.1.2 should have been conducted. The adequacy of the extraction procedure can be assessed only after a more complete characterization of the nature of the DNA occurring in the interfacial fraction. Small variations in the extraction conditions could constitute an important source of experimental error and could exert a profound influence on the $^{3}H/^{14}C$ ratios obtained. It would be reassuring if, after proteinase treatment and hydroxyapatite chromatography, a mild acid hydrolysis (with or without added carrier CH_2O) could be shown to release radio-labeled CH_2O from this material. This would give positive evidence for the existence of chemically-incorporated CH_2O in the IF-DNA fraction.

Exposures to labeled CH_2O were routinely conducted over a period of six hours with rats that had been preexposed to the same concentration of the gas for six hours the previous day. In view of the established temporal changes in cell proliferation as well as the replacement of respiratory epithelial cells by squamous cells during chronic exposure, there is some question whether the results of the acute labeling studies will accurately reflect events occurring during longer-term exposures.

This is particularly important when it is considered that squamous cell carcinoma does not develop until 11 or 12 months into the chronic study and that a large percentage of DNA protein crosslinks are subject to relatively rapid repair. CIIT investigators argue that the short-term studies are the most appropriate models for human exposure since in humans there is no evidence of the marked changes in epithelial cell structure that are observed during the chronic rat studies. On the other hand, it is not really clear as to whether or to what extent CH_2O-DNA interactions differ during the course of chronic exposure.

Thus, the short-term conditions employed to evaluate CH_2O binding may or may not reflect those occurring during chronic exposures, and there is considerable uncertainty in relating the acute binding data directly with the carcinogenic lesions occurring as a result of chronic exposure.

While clearly it is not possible to conduct binding studies throughout the entire period of the chronic test, it would be useful for comparative purposes to have data from animals exposed to CH_2O for a longer period of time. The primary uncertainty associated with the data is that they represent an acute model of a chronic lesion. The extent to which this model is valid remains to be determined.

Information provided by CIIT scientists at the meeting addressed satisfactorily initial questions concerning methodology for determination of $^{3}H/^{14}C$ ratios. Samples of raw data were provided which clearly showed very good counting statistics. The problems, which could potentially arise from quenching, were minimized and controlled through (1) routine use of external standardization (built into the scintillation counter), (2) routine counting of quenched and unquenched standards with samples, and (3) using a relatively constant ratio of scintillation fluid to the aqueous samples. For standardization between experiments, observed $^{3}H/^{14}C$ ratios were normalized to the $^{3}H/^{14}C$ ratio of the gas phase to which the animals were exposed.

There are numerous points at which tritium kinetic isotope effects could influence the disposition of $^{3}H/^{14}C$ dual-labeled CH_2O. For example, there could be a large primary isotope effect on the enzymic oxidation of CH_2O to formate. Smaller secondary isotope effects would influence the addition of nucleophiles to monomeric CH_2O and the reactions by which formate equivalents are incorporated into purine bases. It would, in fact, be amazing if these kinds of isotope effects did not enter into the results. The problems arise because of the complexity of the overall disposition of CH_2O (see Section 3.1.1). This affords the opportunity that under different conditions of administration the relative importance of the various steps will vary. Since the overall (observed) isotope effect will be a composite (a weighted average) of the isotope effects on these individual steps, there is great opportunity for quantitative variation among the individual steps even in the face of an apparently constant value for the $^{3}H/^{14}C$ ratio observed in a particular fraction such as AQ-DNA. This is not to suggest that there are problems of this sort with the data presented, only that there is reason for concern that there might be. Measurement of $^{3}H/^{14}C$ ratios found in specific bases obtained by hydrolyzing AQ-DNA and IF-DNA after both high and low doses of CH_2O would help clarify this situation.

3.3 Identity of Labeled Fractions

This issue/question is addressed under Section 3.1 and the last part of Section 3.2.

3.4 Other Measures of Exposure

Questions/issues Nos. 4 and 7 of Appendix 3 were addressed together and are discussed below.

While a measure of the effective concentration of a chemical at its target site (i.e., the delivered dose) would be preferable to the use of administered dose for purposes of risk assessment, it is questionable whether the DNA-binding data generated by Casanova-Schmitz et al. provide a validated measure of CH_2O concentration in the nasal epithelium of exposed rats. Since the relationship between DNA binding and carcinogenicity have not yet been established for CH_2O, the observations on binding may be of some mechanistic significance. However, while DNA-binding may constitute a satisfactory measure of target site (delivered) dose, its measurement by means of dual-labeled CH_2O incorporation seems unnecessarily sophisticated and complex.

What is really needed as a biochemical dosimeter is some measure of the chemical interaction of CH_2O with intracellular macromolecules other than through metabolic incorporation into nucleotides and/or amino acids. DNA-protein crosslinks, if they could be shown unambiguously to involve methylene links originating from administered CH_2O, would be a valid measure of this. Alternatively, since the *in vitro* experiments show that proteins are far more reactive toward CH_2O than nucleic acids, and since the metabolic incorporation of CH_2O equivalents into proteins is likely to be much less than its metabolic incorporation into nucleic acids, the measurement of CH_2O covalently bound to intracellular proteins could provide a simpler index of

exposure of the cell to CH_2O. Furthermore, validation of these assumptions could easily be accomplished.

While the data provided by the Casanova-Schmitz et al. study may provide some measure of delivered dose, it is not yet clear whether the data are a great deal more useful than measures of administered dose for the purposes of risk assessment. In the absence of DNA binding data obtained following longer periods of exposure, there is no justification for assuming that the short-term data bear any relationship to target-site concentrations likely to be encountered throughout the two-year bioassay.

3.5 Nonlinearity for Crosslinked DNA at Low Doses

3.5.1 Documentation of Nonlinearity for Low Dose Crosslinked DNA

Casanova-Schmitz et al. calculate the amount of covalent binding from equations Nos. 8 and 9 in their Appendix 2. The issue of whether the result actually represents the extent of crosslinked DNA is addressed elsewhere in this report and will not be discussed further here. The authors have assessed the nonlinearity of the dependence of crosslinking on administered dose in two ways.

1. In the discussion section in Casanova-Schmitz et al. there is a comparison between:

   a. The actual values calculated from data at 2 ppm.

   b. The value predicted by interpolating between the results for 6 ppm and the origin. The observed values are 0.022 +/- 0.005 nmol/mg (mean +/- 1 ISE), while the interpolated values are stated to be 0.078 +/- 0.013 (one third of the 6 ppm values, 0.233 +/- 0.023; we note that simple division gives 0.078 +/- 0.0078). The conflict between observed and predicted values is clear.

2. In Appendix 3 of Casanova-Schmitz et al., a more systematic test of proportionality is described. The basic idea is to convert the concentration at each administered dose into an estimated slope by dividing by the dose. If the response were truly proportional to dose, these estimated slopes would all be the same. Therefore, the hypothesis may be tested by examining the consistency of the slopes. Since the standard errors of the estimated slopes are more or less constant, and in particular show no systematic tendency either to increase or decrease with increasing dose, the statistical method of one-way analysis of variance is appropriate and leads to rejection of the null hypothesis of proportionality.

It has been suggested by Cohn (1984) and Cohn et al. (1985) that olfactory IF-DNA may give a better indication of metabolic incorporation than does respiratory AQ-DNA, and that when calculated in this alternative way, the extent of crosslinking becomes proportional to dose. This suggestion has been

disputed by the Casanova-Schmitz et al. authors. However, they argue in their rebuttal that even when calculated in this alternative way, there is still strong evidence of nonlinearity. The comparison between results at 2 ppm and 6 ppm is no longer sharp, and the one-way analysis of variance yields only marginally significant results. However, the linear regression against dose is statistically significant, and the authors correctly point out that this is a more powerful test against alternative hypotheses of the type expected here.

Figure 1 provides a graphical assessment of the proportionality hypothesis. It shows the estimated slopes calculated in both ways with standard errors attached, plotted against dose. The left-hand bar of each pair uses respiratory AQ-DNA as the baseline, while the right-hand bar is based on olfactory IF DNA. Although the nature of the nonconsistency is different for the two alternatives, it is strong in both. From a purely statistical point of view, the weakness of the use of olfactory IF-DNA is that weaker pairing of the data leads to generally larger standard errors, and consequently lower power to detect nonconsistency (nonlinearity of amount of crosslinking).

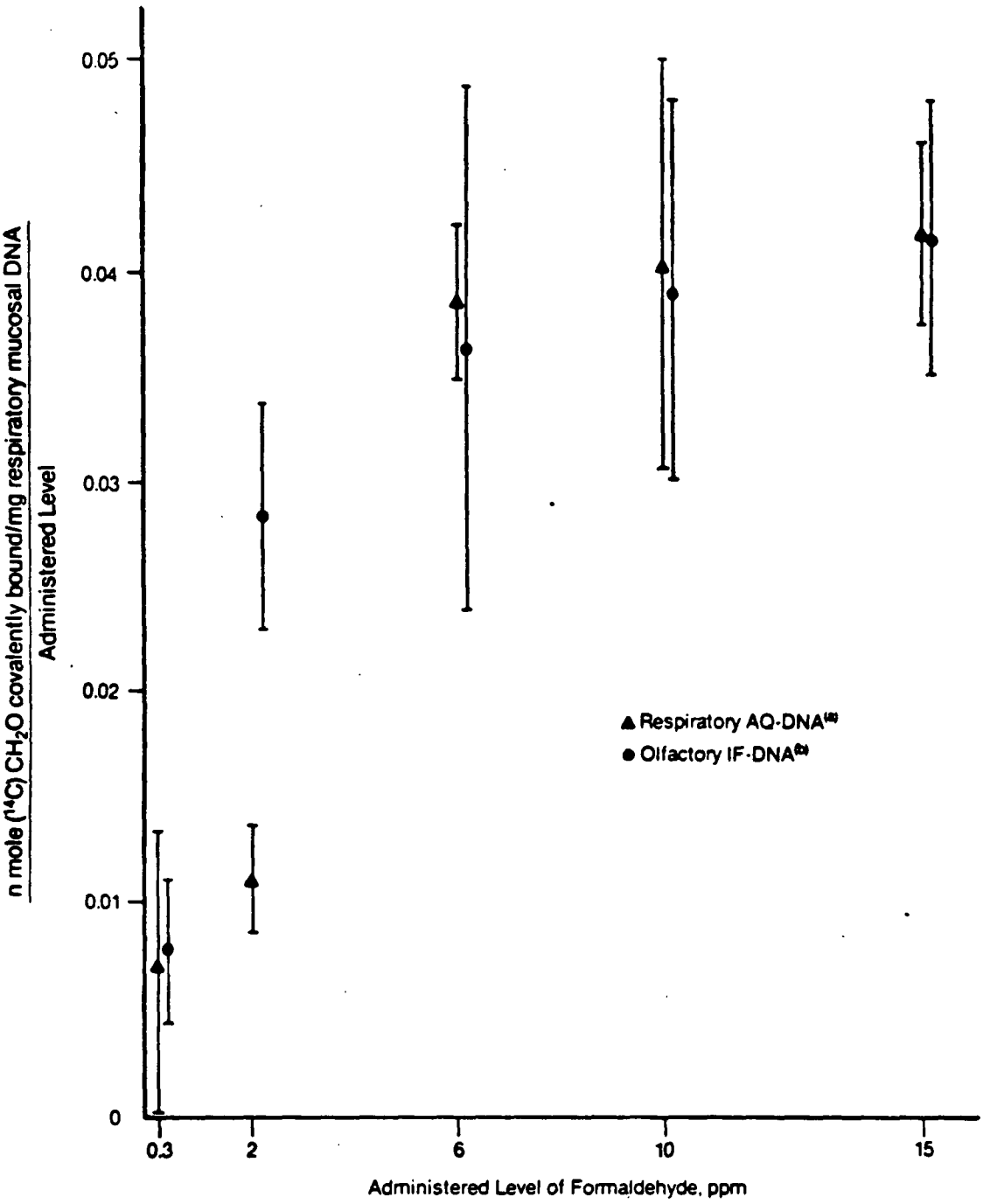
To summarize, in contrast to the claims of Cohn (1984) and Cohn et al. (1985), we find that the nonlinearity of "crosslinked DNA" as a function of administered dose is adequately documented by Casanova-Schmitz et al.

3.5.2 Alternative Explanations for Nonlinearity of Low Dose Crosslinked DNA

Each determination of crosslinked DNA is based on three replications with each replicate based on material from four animals. With such small numbers of measurements, it is always possible that spurious results may be obtained. The calculation of significance levels is the statistician's way of trying to evaluate the weight of evidence in small samples and to avoid being misled by spurious indications. The Casanova-Schmitz et al. authors have been careful to calculate these. However, additional data, especially below 6 ppm, would add considerable substance to the results. There are other mechanisms that might lead to apparent nonlinearity. For instance, suppose that there were a small but constant loss in the measurement of IF-DNA. This would be increasingly important at low doses, and hence, would induce an apparent threshold in the amount of crosslinked DNA. As indicated in Section 3.2, the efficiency of extraction of DNA and the verification of distribution are of prime importance in excluding possibilities such as these.

The authors have suggested that physiological and biochemical defense mechanisms could become less efficient with increasing CH_2O concentrations. We agree that the processes of mucociliary clearance and DNA repair could become saturated as the CH_2O concentration increases, and DNA-protein crosslink formation could therefore increase disproportionately.

Furthermore, the disproportionate increase in $^3H/^{14}C$ ratio with increase in CH_2O concentration might be due to artifactual disturbances in the $^3H/^{14}C$ ratio rather than a true increase in crosslinked DNA-protein in the IF fraction. The effect of CH_2O is somewhat complicated, since CH_2O is known to inhibit DNA synthesis, yet cause an increase in cell turnover in a small



(a) Casanova-Schmitz, M., Starr, T.B., and Heck, H. D'A. (1984) Differentiation Between Metabolic Incorporation and Covalent Binding on the Labeling of Macromolecules in the Rat Nasal Mucosa and Bone Marrow Inhaled (^{14}C)- and (^{3}H) CH_2O. *Toxicology and Applied Pharmacology* 76, 26-44.

(b) Memorandum to Peter W. Preuss from Murray S. Cohn concerning "Health Sciences comments in response to the Environmental Protection Agency's request for information regarding CH_2O..." dated July 16, 1984.

FIGURE 1 ESTIMATED SLOPES FOR METABOLIC INCORPORATION OF RESPIRATORY AQ-DNA AND OLFACTORY IF-DNA

percentage of cells. Inhibition of DNA synthesis would decrease metabolic incorporation but might also decrease sites for adduct formation if these are restricted to the replication fork. More experimentation is thus necessary to determine how inhibition of DNA synthesis and/or increase in cell turnover influence the $^{3}H/^{14}C$ ratio in IF-DNA.

Cell death might also be involved in the nonproportional response in the low dose range. Thus, if cell death occurs, nucleic acids are released and degraded. The subsequent increase in nucleotides, nucleosides, purines and pyrimidines could inhibit de novo nucleotide synthesis from CH_2O by feedback regulation as well as increase the deoxynucleotide pools, thus, diluting out radioactivity incorporated into DNA. A decrease in DNA synthesis from labeled CH_2O, in the absence of an effect on adduct formation, would tend to increase the $^{3}H/^{14}C$ ratio disproportionately at higher CH_2O concentrations.

3.6 Sensitivity of the Study Conclusions to Statistical Analysis

The statistical methods for the comparison of AQ- and IF-DNA in terms of incorporated concentrations of (^{14}C) CH_2O were described as a two-way analysis of variance followed by paired t-tests for individual concentrations. The two factors in the analysis of variance were concentrations of CH_2O and type of DNA: IF and AQ. It is not clear if the pairing of IF- and AQ-DNA determinations were taken into account in the two-way analysis of variance. Moreover, the lack of homogeneity of variance between the low and high concentrations was apparently not taken into account in the analysis; neither were ordered alternatives over the concentrations when the two-way analysis of variance led to statistically significant tests. Thus, more powerful procedures could have been employed to examine the differences in concentrations of (^{14}C) CH_2O between IF- and AQ-DNA over CH_2O concentrations.

An examination of the data on respiratory DNA reveals that there is a statistically verifiable increase in the concentration of bound (^{14}C) CH_2O per mg DNA over CH_2O concentration as concluded in the paper. These comments also apply to the comparisons of $^{3}H/^{14}C$ ratios between IF- and AQ-DNA as a function of CH_2O concentrations. Thus, the overall conclusions on the comparisons of IF- and AQ-DNA as a function of CH_2O concentrations hold even though the statistical analysis could be strengthened.

In their comparisons of IF- and AQ-DNA with paired t-tests at separate CH_2O concentrations, Casanova-Schmitz et al. concluded that the bound (^{14}C) CH_2O concentrations did not differ significantly at 0.3 and 2 ppm. The power of these paired t-tests is low for these CH_2O concentrations because of the small sample sizes at individual concentrations relative to the coefficient of variability. Although this is not critical with respect to the pattern of the IF- and AQ-DNA data over the CH_2O concentrations used in the experiment, it does limit the extent to which inferences can be made about the responses to low concentrations for purposes of identifying no-response levels or making low-dose extrapolations for risk assessment.

3.7 Adequacy of the Measure of Exposure

This question/issue was fully addressed along with No. 4 in Section 3.4 above.

3.8 Utility of the Study in the Quantitative Risk Assessment of CH_2O

The panel recognized this study as an important step toward attempting to assess the intracellular dose delivery of externally applieded CH_2O. These efforts should be continued toward the ultimate goal of improving the assessment of risk. At its present level of development and validation, however, the study does not represent an adequate basis for quantitative risk assessment. First, the problem of proper validation of the experimental methodologies must be accomplished to assure that CH_2O-DNA-protein complexes are identified properly, and that the experimental assumptions are valid. The evidence is not sufficiently strong at this time to reject the linear dose extrapolation model. Second, the selection of a single intracellular target is complicated by the nature of binding processes with DNA and could be augmented appropriately by the additional analysis of binding to intracellular proteins. Third, and perhaps most important, the selection of the acute model may not be entirely appropriate since it is the chronic dosimetry that is most relevant to risk assessment. The factors which account for nonlinearity of dose delivery may well vary considerably (in either direction) as a result of chronic treatment.

This study is an important first step toward the introduction of intracellular dosimetry into the risk assessment process. The continuation and extension of these investigations should be encouraged.

4.0 CONCLUSIONS AND RECOMMENDATIONS

The following summarizes the conclusions and recommendations developed by the review team during the December 2 and 3, 1985 meeting. These conclusions and recommendations have undergone final review by each of the experts participating in the meeting.

1. Some doubt still remains as to the validity of the assumptions which form the basis for distinguishing metabolically incorporated and crosslinked (or adducted) CH_2O, i.e., $^3H/^{14}C$ in DNA.

2. Experimental methods and controls were adequate with respect to monitoring the CH_2O administration and analysis of dual-labeled materials. However, the chloroform/iso-amylalcohol/phenol extraction for DNA and DNA crosslinked to proteins was not validated in terms of the identities of materials separated nor the overall efficiency and consistency of extraction. The occurrence of underlying variability incorporation due to kinetic isotope effects on the disposition of tritiated CH_2O can neither be assessed nor discounted.

3. Sufficient documentation is still unavailable to state unequivocably that all the crosslinked DNA-protein complexes occur in the IF-DNA fraction.

4. There remains a need for an effective biochemical dosimeter to measure the dose of CH_2O delivered to the cells of the nasal epithelium. The data provided by Casanova-Schmitz et al. are not considered a sufficiently well-validated measure of this parameter.

5. The nonproportionality of the calculated concentration of bound ^{14}C (CH_2O)-DNA as a function of the administered dose is documented adequately. Whether the nonproportionality truly reflects crosslink formation or is due to the small sample size, to a constant loss in the recovery of IF-DNA, or to artifactual disturbances in the $^3H/^{14}C$ ratio remains to be elucidated.

6. The increase in concentration of bound ^{14}C with the concentration of CH_2O is well documented, as is the increase in the difference in the $^3H/^{14}C$ ratio between IF- and AQ-DNA. The power of separate comparisons for the 0.3 and 2 ppm doses is low because of small sample size relative to the coefficient of variation. This limits the potential for inferences about no-response levels and low-dose extrapolations.

7. The study of Casanova-Schmitz et al. is an important first step toward quantitative assessment of the intracellular level of CH_2O in the nasal mucosa of the rat following inhalation exposure. At its present level of validation, however, it does not provide a basis for such quantitation. Furthermore, the selection of an acute study model may not be appropriate to the assessment of chronic toxicity.

APPENDIX 1

DOCUMENTS PROVIDED TO REVIEWERS

1. Preliminary Assessment of Health Risks to Garment Workers and Certain Home Residents Exposure to Formaldehyde. EPA Draft Report. May 31, 1985.

2. Casanova-Schmitz, M., Starr, T.B., and Heck, H. D'A. (1984) Differentiation Between Metabolic Incorporation and Covalent Binding on the Labeling of Macromolecules in the Rat Nasal Mucosa and Bone Marrow Inhaled (^{14}C)- and (^{3}H) CH_2O. Toxicology and Applied Pharmacology 76, 26-44.

3. Cohn, M.S., DiCarlo, F.J., and Turturro, A. (1985) Letter to the Editor. Toxicology and Applied Pharmacology. 77, 363-364.

4. _____. (1985) Letter to the Editor. Toxicology and Applied Pharmacology. 77, 365-368.

5. _____. (1985) Letter to the Editor. Toxicology and Applied Pharmacology. 77, 358-361.

6. Selected comments pertaining to the use of the CIII "effective dose" experiment. . .

7. Memorandum to Peter W. Preuss from Murray S. Cohn concerning "Health Sciences comments in response to the Environmental Protection Agency's request for information regarding CH_2O. . ." dated July 16, 1984.

APPENDIX 2

ADDITIONAL REFERENCES RELATING TO EXPERT REVIEW OF PHARMACOKINETIC DATA: FORMALDEHYDE

1. Starr TB, Buck RD. 1984. The importance of delivered dose in estimating low-dose cancer risk from inhalation exposure to formaldehyde. Fund. Appl. Tox. 4:740-753.

2. Comments to the EPA Science Advisory Board by CIIT scientists regarding the EPA draft entitled "Preliminary Assessment of Health Risks to Garment Workers and Certain Home Residents from Exposure to Formaldehyde." June 21, 1985.

3. Comments to the EPA Science Advisory Board by Dr. James A. Swenberg regarding the EPA draft entitled "Preliminary Assessment of Health Risks to Garment Workers and Certain Home Residents from Exposure to Formaldehyde." July 9, 1985.

4. Comments to the EPA Administrator by the Environmental Health Committee of EPA's Science Advisory Board regarding the EPA draft entitled "Preliminary Assessment of Health Risks to Garment Workers and Certain Home Residents from Exposure to Formaldehyde." Undated.

APPENDIX 3

QUESTIONS/ISSUES ON FORMALDEHYDE TO BE ADDRESSED BY EXPERT PANEL

1. Are the assumptions which form the basis for distinguishing between metabolically incorporated and crosslinked formaldehyde adequately supported?

2. The appropriateness or limitations of the experimental methodology used.

3. What do the measurements taken in the Casanova-Schmitz study represent-i.e., is there ambiguity in the identity of the various labeled fractions? Do the data establish that the IF fraction consist of crosslinked DNA?

4. Are there any other data in the study that could be used as a measure of exposure in addition to the crosslinked DNA?

5. Is the nonlinearity for crosslinked DNA at low doses adequately documented? Are there alternative explanations for these observations?

6. What is the sensitivity of the conclusions of the study to both experimental error and the statistical treatment of the data?

7. Does the study give a better measure of exposure than the "applied dose" for the second day post exposure; and if so, does it also give a better measure of the dose during the two-year bioassay?

8. Conclusions the experts can draw concerning the utility of the study (and any underlying data) in the quantitative risk assessment of formaldehyde.

APPENDIX 4

INFORMATION/CLARIFICATION REQUESTED FROM CIIT
BY EXPERT PANEL

1. Would like to see information on the methodology and equipment used for scintillation counting. Needs information on quench correction and the methods used to calculate DPMs.

   Would also like to see an example of the raw data used to calculate DPM.

2. Would like to review raw data used to perform two-way analysis of variance between aqueous phase and interfacial DNA with respect to their incorporation concentrations of (^{14}C) CH_2O equivalents or with respect to their $^{3}H/^{14}C$ ratios at different concentrations of CH_2O.

3. Request copies of the following references:

   a. Swenberg, J. A., Gross, E. A., Martin, J., and Popp, J.A. (1983a). Mechanisms of formaldehyde toxicity. In Formaldehyde Toxicity (J. E. Gibson, ed.), pp. 132-147. Hemisphere, Washington, D.C.

   b. Swenberg, J. A., Gross, E. A., Randall, H. W., and Barrow, C. S. (1983b). The effect of formaldehyde exposure on cytotoxicity and cell proliferation. In Formaldehyde: Toxicology, Epidemiology and Mechanisms (J. J. Clary, J. E. Gibson, and R. S. Waritz, eds.), pp. 225-236, Dekker, New York.

APPENDIX 5

TR-835-19

LIST OF PARTICIPANTS

Expert Review of Pharmacokinetic Data: Formaldehyde

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APPENDIX 6

TR-835-18

AGENDA

EXPERT REVIEW OF PHARMACOKINETIC DATA: FORMALDEHYDE

December 2-4, 1985
Chamber C, Greenbrier Ballroom
Sheraton University Center
Durham, NC

Meeting Chairperson: Dr. Lemone Yielding

Time	Agenda Item	Individual
Monday, December 2, 1985		
9:00 a.m.	Informal Technical Discussions[a]	
12:00 noon	Break	
1:00 p.m.	Welcome	
	1. Administrative Announcements	D. Meckley
	2. Summary of EPA's Needs	W. Farland
1:45 p.m.	Meeting Objectives	J. Glennon
2:00 p.m.	Chairman's Opening Comments	L. Yielding
2:30 p.m.	Input From CIIT	Dr. Heck
4:30 p.m.	Finalize List of Questions and Issues	L. Yielding
5:30 p.m.	Break	
7:00 p.m.	Discussion of Questions and Issues	L. Yielding
	1. Discussion	
	2. Consensus	
	3. Assignment of Draft Report Authors[b]	
9:00 p.m.	Adjourn for Day	

continued-

(a) Optional for those individuals arriving December 1, 1985.
(b) Selected authors of Draft Report sections may adjourn to prepare rough draft or entire meeting may adjourn to prepare Draft Report sections as determined by the participants.

Agenda - continued

Time	Agenda Item	Individual
Tuesday, December 3, 1985		
8:00 a.m.	Administrative Announcements	D. Meckley
8:10 a.m.	Chairman's Comments	L. Yielding
8:20 a.m.	Discussion of Questions and Issues	L. Yielding
	1. Discussion 2. Consensus 3. Assignment of Draft Report Authors	
12:00 noon	Break	
1:00 p.m.	Discussion of Questions and Issues - continued	L. Yielding
3:00 p.m.	Meeting Status Summary	L. Yielding J. Glennon
	1. Questions and Issues Resolved 2. Remaining Action Items 3. Revision of Agenda/Schedule	
4:00 p.m.	Discussion of Questions and Issues - continued	L. Yielding
5:00 p.m.	Adjourn for Day	
Wednesday, December 4, 1985		
8:00 a.m.	Administrative Announcements	D. Meckley
8:10 a.m.	Draft Report Status	L. Yielding
8:20 a.m.	Preparation of Draft Report	L. Yielding
	1. Complete Action Items 2. Review/Discuss Draft Report Sections 3. Revise Draft Report Sections	
12:00 noon	Break	
1:00 p.m.	Preparation of Draft Report - continued	L. Yielding
4:00 p.m.	Final Review of Draft Report Status	L. Yielding J. Glennon
5:00 p.m.	Adjourn	

APPENDIX 1: EXPERT PANEL REPORT ON HCHO PHARMACOKINETIC DATA AND CIIT RESPONSE

Chemical Industry Institute of Toxicology

CIIT

P. O. Box 12137
Research Triangle Park,
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President, Robert A. Neal, Ph.D.
Vice President, Director of Research, James E. Gibson, Ph.D.
Vice President, Administration and Secretary, Donald A. Hart, Ed.D.

February 4, 1986

Dr. William H. Farland
Deputy Director
Health and Environmental
Review Division
Office of Pesticides and
Toxic Substances
U. S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

Dear Dr. Farland:

Enclosed are detailed comments on the document "Expert Review of Pharmacokinetic Data: Formaldehyde" which was authored at the meeting in Research Triangle Park on December 2-4. I have also sent copies of these comments to the other members of the committee inviting their individual or collective comments. We would also welcome any comments you personally might have concerning points raised in this critique.

Sincerely,

Robert A. Neal
President

RAN:ewb

Enclosure

COMMENTS ON THE FINAL REPORT OF THE PANEL REVIEWING THE CIIT PHARMACOKINETIC DATA ON FORMALDEHYDE

M. Casanova, T. B. Starr, and H. d'A. Heck

We have carefully examined the final report of the Panel reviewing the CIIT pharmacokinetic data on formaldehyde. We find many of their concerns to be without merit, and we disagree strongly with their conclusions. A detailed justification for this assessment of the Panel's report is given below.

3.1.1. Metabolic Incorporation versus Adduct Formation or Crosslinked CH_2O

The Panel states that the "interpretation of the $^3H/^{14}C$ ratio of the DNA due to metabolic incorporation of [^{3}H]- and [^{14}C]CH_2O] is very complex" owing to the fact that "relative reaction rates and pool sizes are likely to vary under different conditions". Hence, the Panel argues that it is necessary to isolate the DNA bases by HPLC and to determine the isotope ratios of the isolated bases. The Panel did not mention that we have already undertaken such studies, although we informed the Panel of this on December 2, 1985, when we had our meeting with them. The Panel did not request details of our studies either at the meeting or subsequent to the meeting, although we volunteered to provide any information requested. It is surprising to us, therefore, that they called in their report for HPLC studies to be done.

For the record, we would like to provide the essential results of our HPLC analyses of DNA. The DNA samples that were analyzed were obtained from rats that had been exposed (6 hr) to 0.3, 2, or 6 ppm of [^{3}H]- and [^{14}C]formaldehyde. These were the same samples that had previously been used for determinations of covalently bound CH_2O in DNA (Casanova-Schmitz et al., 1984). Sufficient AQ DNA remained from those samples for analysis by HPLC. However, only one IF DNA sample remained from the initial experiments, and that sample was obtained from rats exposed at 6 ppm.

The major UV-absorbing peaks from respiratory mucosal AQ DNA samples eluted at the same positions as authentic purine and pyrimidine deoxyribonucleoside standards. Calculation of the base compositions of the DNA samples was performed after calibration of the UV monitor with nucleoside standards. The values obtained for the base compositions of the DNA samples agreed well with one another and with the base compositions reported in the literature; obs.: deoxyadenosine (dAdo), 28.9 ± 0.3 %; deoxycytidine (dCyd), 21.3 ± 0.4 %; lit. values (Shapiro, 1968): dAdo, 28.8 ± 0.7 %; dCyd, 20.5 ± 0.5 %. This signifies that the base composition of the AQ DNA is the same as that of the total rat DNA. The nucleosides, deoxyguanosine (dGuo) and thymidine (dThd), were not completely resolved in the chromatography, hence, their individual base compositions were not calculated.

As expected (Casanova-Schmitz et al., 1984), most of the radioactivity in the AQ DNA from the respiratory mucosa eluted at the positions of the normal deoxyribonucleosides, dGuo, dThd, and dAdo, implying that the labeling of the AQ DNA was primarily caused by normal metabolic incorporation. In three AQ

DNA samples from rats exposed to 6 ppm of CH_2O, the percentage of the total ^{14}C that eluted at the positions of the normal nucleosides was 100%, 96%, and 90%, respectively. In two of the three samples, small amounts of radioactivity eluted prior to the major peaks, but no radioactivity eluted after dAdo in any sample. A late-eluting radioactive peak would be expected for 6-hydroxymethyl-deoxyadenosine, a postulated adduct of formaldehyde with DNA (Beland et al., 1984). If such an adduct were formed, it did not remain in our DNA samples until the time of analysis.

The $^3H/^{14}C$ ratios of the major peaks were consistent with normal metabolic incorporation. The isotope ratios of the deoxyribonucleosides did not vary measurably with concentration over the range 0.3 to 6 ppm. We observed that the $^3H/^{14}C$ ratio of dAdo (0.55) was higher than that of dGuo (0.25). A higher isotope ratio for dAdo than for dGuo is consistent with the known pathway of formaldehyde incorporation via tetrahydrofolate into positions 2 and 8 of inosine monophosphate (IMP), the precursor of both GMP and AMP. The conversion of IMP to GMP involves the introduction of an oxygen atom at position 2 of the purine ring (with consequent loss of ^3H at this position). The conversion of IMP to AMP does not involve a corresponding loss of ^3H at position 2. Thus, AMP should have a higher $^3H/^{14}C$ ratio than GMP, as observed experimentally.

We observed no labeling of dCyd in our chromatograms. This implies that the labeling of dThd ($^3H/^{14}C = 0.4$) was due only to labeling at the 5-methyl position, which results from transfer of the methylene carbon atom from N^5,-N^{10}-methylene-tetrahydrofolate to deoxyuridine monophosphate.

The $^{3}H/^{14}C$ ratios of the minor peaks seen in two of the three chromatograms were low (ranging from 0.3 to 0.4), resembling the ratios seen in the major peaks. This suggests that the minor peaks of radioactivity were not due to covalent adducts, which would be expected to have higher $^{3}H/^{14}C$ ratios than those seen in the normal bases. It is likely that at least some of the minor radioactive peaks in the AQ DNA samples were due to slight contamination of the DNA with RNA. This hypothesis is supported by the observation that minor peaks eluted at positions similar to those obtained using ribonucleoside standards, adenosine and guanosine. It is also possible that a minor peak resulted from deamination of dAdo to deoxyinosine, which might have occurred if the alkaline phosphatase used in the hydrolysis of DNA had been contaminated with adenosine deaminase (Gehrke et al., 1982). (No loss of ^{3}H would occur in this reaction, therefore, the $^{3}H/^{14}C$ ratio would remain unchanged.) Finally, a minor peak may have been due to 5-methyl-deoxycytidine (5-MedCyd), a normal constituent of rat DNA accounting for about 1% of the bases (Shapiro, 1968). The presumed labeling of the 5-MedCyd would be expected to occur via transfer of a methyl group from S-adenosylmethionine to dCyd in DNA (Kornberg, 1980). This methyl group could be labeled, since methionine might be synthesized in small amounts from methyl-tetrahydrofolate, although methionine is usually considered to be an "essential" amino acid.

It should be noted that even if one assumes the most extreme case that 10% of the radioactivity in the AQ DNA were due to RNA, the error in estimating the $^{3}H/^{14}C$ ratio of the AQ DNA would be only 1%, due to the similar isotope ratios of RNA and AQ DNA. Moreover, since RNA has a higher specific activity than DNA (Casanova-Schmitz et al., 1984), the actual contamination of the DNA

by RNA would be less than 4% in the most extreme case. Errors of these magnitudes are completely negligible in our calculations of covalent binding.

A single IF DNA sample from rats exposed to 6 ppm of [^{3}H]- and [^{14}C]CH_2O was also analyzed by HPLC. In this sample, one additional peak was seen that was not present in any AQ DNA sample. This peak eluted very early in the chromatogram and had an apparent $^3H/^{14}C$ ratio > 1.0. Such a peak could conceivably be incompletely digested DNA containing covalently bound CH_2O, the hydrolysis of which was prevented by DNA-amino acid or DNA-peptide cross-linking. This interpretation is, of course, tentative, and additional studies are needed to test this hypothesis. As in the AQ DNA, there were no peaks eluting after dAdo.

It is difficult to unequivocally identify minor peaks seen in HPLC, owing to the low levels of radioactivity and the small amounts of DNA in the IF DNA samples. After enzymatic hydrolysis, each of the major metabolically-labeled nucleoside peaks in the IF DNA contained only about 100 to 200 dpm, and the total radioactivity in the unidentified early-eluting minor peak in IF DNA with the high $^3H/^{14}C$ ratio was only 80 dpm. Thus, owing to the small amount of DNA obtainable from the rat nasal mucosa, the low level of radioactivity in the IF DNA that was due to cross-linking, and the present lack of information concerning the detailed structures of the DNA-protein cross-links, we doubt that much more information than we have already obtained would result from continuing or extending the HPLC studies, despite the recommendation of the Panel. At least the <u>qualitative</u> outcome of such studies has already emerged from our work.

It should be emphasized that the results of our HPLC analyses of DNA samples collected from exposed rats are in full agreement with our conclusions that AQ DNA does not contain covalently bound CH_2O, i.e., that the labeling of AQ DNA is due to metabolic incorporation, whereas the labeling of IF DNA is caused both by metabolic incorporation and covalent binding. We are satisfied that the concerns of the Panel concerning the source of the label in AQ and IF DNA has been adequately addressed by our research.

3.1.2 Crosslinked CH_2O Located Exclusively in the Interface (IF) DNA

As discussed above, there was no HPLC evidence for either adducts or cross-links in the AQ DNA at either 0.3, 2, or 6 ppm. In contrast, the IF DNA at 6 ppm did provide such evidence.

The Panel states in this section that "the efficiency of extraction of the DNA from respiratory epithelium under conditions of CH_2O dosing (at various levels) should have been determined". Detailed information concerning our extraction efficiencies had not been requested by the Panel prior to the meeting, nor was it requested subsequent to the meeting. For the record, it should be noted that the average DNA yield per mg wet weight of respiratory mucosal tissue (4.20 ± 0.10 μg; mean ± SE, n = 30) did not vary significantly with concentration ($p = 0.473$; one-way ANOVA) over an airborne concentration range of 0.3 to 15 ppm and exposure times of either 3 or 6 hr. Thus, the possibility of a dose-dependent change in the recovery of DNA is ruled out by our data.

Regarding the question of the efficiency of our extraction procedure for DNA, it should be noted that we used the same extraction procedure to isolate DNA from rat liver nuclei treated *in vitro* with formaldehyde. The yield of highly purified DNA (chromatographed on hydroxyapatite and washed by ultrafiltration) that we obtained was 0.82 ± 0.02 mg/g of liver. This result compares extremely well with the total amount of DNA (unpurified) reported to be present in rat hepatic nuclei (0.83 ± 0.03 mg/g) (Blobel and Potter, 1966). The approximately five-fold higher yield of DNA that we obtained from the nasal respiratory epithelium (see preceding paragraph) indicates that the percentage of the total tissue weight that is due to DNA is significantly higher in the nasal mucosa than in the liver.

3.2 Experimental Methodology Limitations

The Panel again raises questions about the extraction efficiency of the DNA, and it asserts that the amount of DNA in the IF fraction will vary with the extraction conditions employed. However, the extraction conditions (volumes, buffers, pH, ionic strength, temperature) were carefully held constant throughout the experiment. We were well aware of the importance of reproducibly recovering a constant amount of DNA in all experiments. Consequently, the same individuals did all of the experiments, and the experiments were always performed with great care. From the inhalation exposure to the final extraction of DNA, the experiments were carried out on the same day using the same protocol. No samples were ever stored. The constancy of the DNA yield with concentration noted above is clear evidence that our concerns (and those of the Panel) were properly addressed *from the beginning*. Therefore, the as-

sertion of the Panel of a possibly varying DNA yield with "variations in the extraction conditions" is contradicted by the evidence.

The question of the interpretation of acute vs. long-term exposure has been addressed numerous times. The essential point is that at low formaldehyde concentrations, i.e., those concentrations to which humans are normally exposed, the transition from normal respiratory to squamous epithelium does not occur. Therefore, covalent binding studies in normal respiratory epithelial cells are highly relevant to risk assessment, where risk is defined as the possibility of covalent reaction with DNA (which may or may not lead eventually to cancer) under normal human exposure situations. The high-concentration, long-term exposure studies do not reveal what occurs biologically at low concentrations, since the cell structure and tissue morphology has been radically altered. Indeed, one of the panelists, Dr. Wilkinson of Cornell, remarked at our meeting on December 2 that such high-dose exposures can well be considered to exceed the "maximum-tolerated-dose".

The final point raised by the Panel in this section is the question of isotope effects in the oxidation of $[^{3}H]$- and $[^{14}C]CH_2O$. At the time of the meeting on December 2 we had already begun studies of possible isotope effects in either the covalent binding or oxidation of CH_2O. These studies were started for reasons other than those raised by the Panel, however, the isotope effect studies were only in preliminary stages in December, and, consequently, the results that we have now obtained could not be given to the Panel. We would like, therefore, to present these results in this document.

First, with regard to covalent binding of formaldehyde, we have examined the $^{3}H/^{14}C$ ratio of the IF DNA recovered from freshly isolated hepatic nuclei incubated in vitro with [^{3}H]- and [^{14}C]CH_2O. The IF DNA isolated from such nuclei was heavily labeled with ^{3}H and ^{14}C, whereas the AQ DNA had practically no radioactivity, consistent with our interpretation that the IF DNA, and not the AQ DNA, contains covalently bound CH_2O. Furthermore, the percent IF DNA as well as the specific activity of the IF DNA increased with increasing concentrations of CH_2O and with increasing times of reaction. The isotope ratio of the IF DNA relative to that of the reaction solution was approximately 1.034 ± 0.009 (mean ± SE, n = 6), indicating an extremely small isotope effect favoring the binding of [^{3}H]CH_2O over that of [^{14}C]CH_2O to DNA. An isotope effect of this small magnitude is negligible insofar as our calculations of covalently bound CH_2O in DNA are concerned.

Second, with regard to metabolic oxidation, we determined the $^{3}H/^{14}C$ ratio of [^{3}H]- and [^{14}C]CH_2O at various times during in vitro incubations of selected concentrations of labeled formaldehyde with freshly isolated homogenates of the rat respiratory mucosa and NAD^+ (1 mM). In most cases, the reaction solutions also contained glutathione (GSH), since the principal enzyme responsible for CH_2O oxidation, formaldehyde dehydrogenase (FDH), is a GSH-requiring enzyme. We observed that the oxidation of [^{3}H]- and [^{14}C]CH_2O catalyzed by FDH occurs with a significant isotope effect. The rate of oxidation of [^{14}C]-CH_2O was approximately 1.82-fold faster than that of [^{3}H]CH_2O, indicating that the hydride transfer step in the GSH-dependent oxidation of CH_2O to HCOOH catalyzed by FDH is at least partially rate-limiting in CH_2O oxidation. The magnitude of the isotope effect was independent of the CH_2O concentration over

a 100-fold concentration range (from 0.1 to 11 μM). Furthermore, we found that aldehyde dehydrogenase exhibits a similar isotope effect to that of FDH in its oxidation of CH_2O to HCOOH, which is GSH-independent.

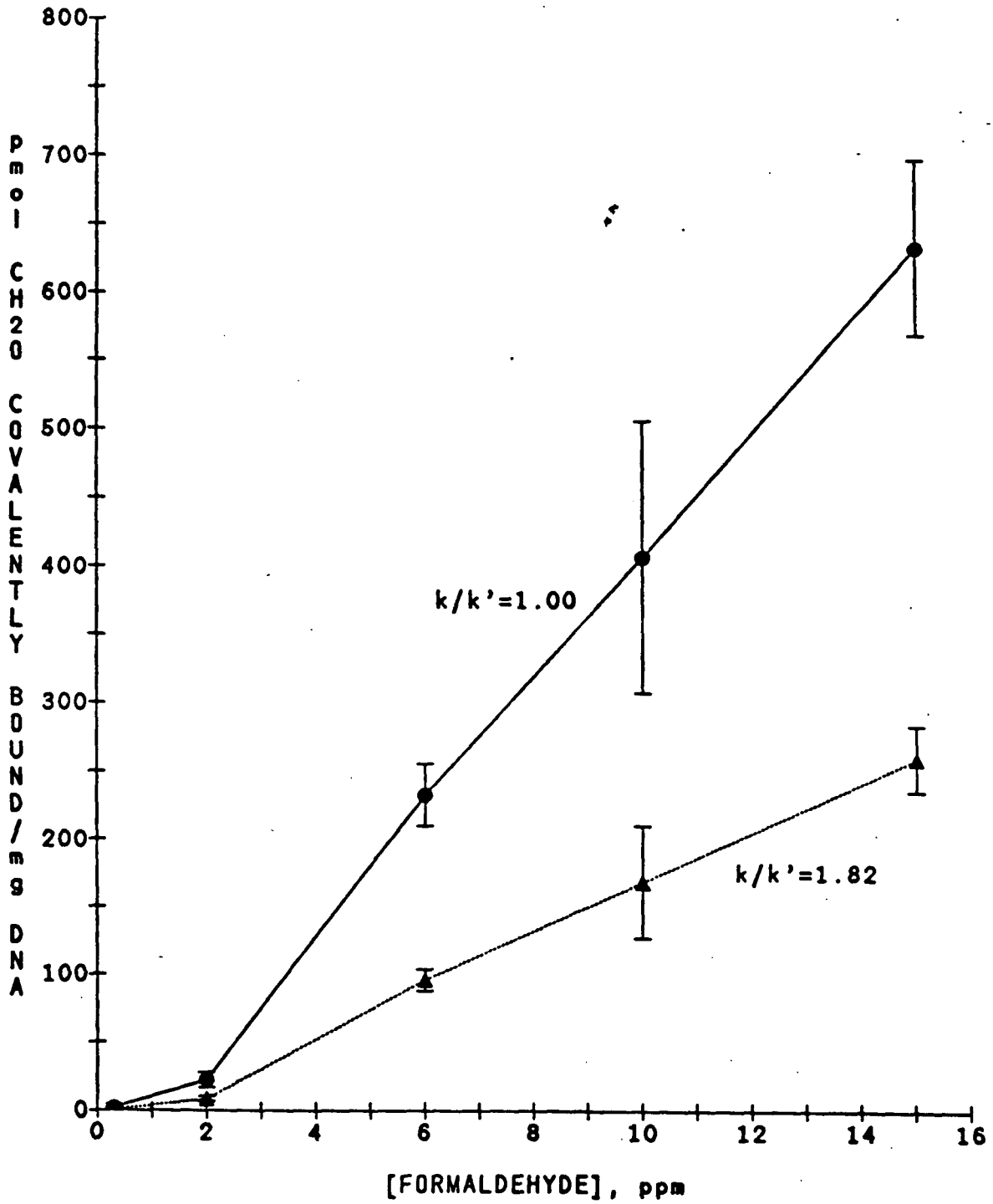
Since an isotope effect in CH_2O oxidation has been demonstrated to occur *in vitro*, it can be presumed that it also occurs *in vivo*. An isotope effect occurring in the oxidation of CH_2O to HCOOH *in vivo* would result in an effective "enrichment" of $[^3H]CH_2O$ relative to $[^{14}C]CH_2O$ in the residual (unoxidized) CH_2O in the nasal mucosa. The $^3H/^{14}C$ ratio of the unoxidized CH_2O would, therefore, be greater than that of the inhaled gas. In the calculation of covalently bound CH_2O in DNA (Casanova-Schmitz *et al.*, 1984), it was implicitly assumed that the $^3H/^{14}C$ ratio of CH_2O in the nasal mucosal cells was identical to that of the gas. It is now recognized that this assumption may be incorrect. The assumption was made because of the practical impossibility of directly measuring the $^3H/^{14}C$ ratio of unmetabolized CH_2O in the cells of the nasal mucosa.

It can be readily shown that an enrichment of $[^3H]CH_2O$ relative to $[^{14}C]$-CH_2O in the cells, resulting from an isotope effect in oxidation, leads invariably to an *overestimate* of the amount of CH_2O covalently bound to DNA, when the calculation of covalent binding is done using our published equations (Casanova-Schmitz *et al.*, 1984). Thus, by implicitly assuming no isotope effect in CH_2O oxidation, our calculation yielded an *upper limit* on the amount of CH_2O bound to DNA. Conversely, by assuming the isotope effect in CH_2O oxidation to be maximal, *i.e.*, that it occurs with an isotope effect of 1.82 independent of the CH_2O concentration, we can calculate a *lower limit* for co-

valent binding of CH_2O to DNA. (The reason that 1.82 represents a maximal isotope effect is that the other routes of CH_2O elimination from cells, such as covalent binding, diffusion out of the cells, or reduction to methanol, do not involve breakage of the ^3H-C bond, and, therefore, they would all show smaller isotope effects than that observed in oxidation. Thus, the assumption of a maximal isotope effect is equivalent to assuming that metabolism is the only route of elimination.) The results of such calculations are shown in Figure 1.

The upper curve in Figure 1 is the same curve as that previously published by us (Casanova-Schmitz et al., 1984), which assumed no isotope effect in CH_2O oxidation. The lower curve is that which would result if the isotope effect had its maximal value. Clearly, an overestimate of covalent binding occurs at all concentrations. However, the fundamental shape of the curves, i.e., their significant departure from linearity at low CH_2O concentrations, is unaffected by the isotope effect in CH_2O oxidation.

In reality, by assuming that an isotope effect occurs in vivo in the oxidation of CH_2O, the curve becomes, if anything, even more nonlinear than was the case before the isotope effect was recognized to occur. This is because the enrichment of ^3H relative to ^{14}C in the residual (unoxidized) CH_2O is greatest under conditions in which the metabolism of CH_2O to HCOOH is most nearly complete, i.e., at low airborne concentrations, and is smallest under conditions in which the metabolism of CH_2O to HCOOH is least complete, i.e., at high airborne concentrations (Melander and Saunders, 1980). Therefore, the overestimate of the amount of CH_2O bound to DNA is greatest at low concentra-



Amounts of CH2O covalently bound to rat nasal mucosal DNA. Upper and lower curves represent yields of covalently bound CH2O assuming either no isotope effect or a maximal (1.82) isotope effect in the oxidation of CH2O by FDH.

Fig. 1

tions and is smallest at high concentrations, causing the curve to become even more nonlinear than before. We would expect, therefore, that the true covalent binding curve should approximate the lower curve of the two shown in Figure 1 at low CH_2O concentrations, and it should approach more closely to the upper curve at high CH_2O concentrations. We conclude that low-dose nonlinearity in the binding of CH_2O to DNA is supported rather than disproved by our finding of an isotope effect in CH_2O oxidation.

3.3 Identity of Labeled Fractions

This issue is addressed in section 3.1 and 3.2.

3.4 Other Measures of Exposure

The Panel remarks that our methods appear to be "unnecessarily sophisticaed and complex". We feel that our methods, far from being complex, are relatively simple. DNA was isolated and purified using established (hydroxyapatite) techniques, and the resulting DNA was counted for radioactivity. Certainly, the use of dual isotopes for metabolic studies is not new. The criticism of our methods by the Panel as being too "sophisticated" is not justified in our opinion.

The Panel suggests measuring covalent binding to intracellular proteins as a "simpler index" of molecular dosimetry. We would ask four questions: (1) Which intracellular proteins would they recommend? (2) What evidence is there that covalent binding to proteins is related to mutagenesis or to cancer? (3)

How would they deal with the issue of protein turnover? (4) How would they correct for labeling caused by metabolism? Clearly, their proposal requires selecting a protein, or a group of proteins, that would be used to monitor CH_2O binding. Such proteins would have to be quantitatively purified from the other proteins in the nasal mucosa, and their rate of turnover would have to be separately determined. Labeling due to metabolism would have to be differentiated from that due to covalent binding. Finally, after solving these extremely challenging experimental problems, it would have to be assumed that covalent binding to these proteins (presuming that such binding could be shown to occur) is somehow related to the initiation of cancer. These critical problems and assumptions clearly mean that intracellular protein binding is not a "simpler index" than DNA binding for molecular dosimetry purposes.

Furthermore, we now have strong evidence that protein binding in vivo primarily involves the extracellular proteins. We informed the Panel that when rats were pretreated with phorone, a GSH depleting agent, there was a marked increase in the amount of CH_2O covalently bound to DNA, as would be expected if oxidation of CH_2O to HCOOH were a major defense mechanism (Casanova-Schmitz and Heck, 1985). In contrast, there was no detectable increase in the amount of CH_2O covalently bound to proteins as a result of phorone pretreatment. Therefore, GSH depletion was ineffective in enhancing the binding of CH_2O to proteins, suggesting that the proteins to which CH_2O is bound are not protected by metabolism. One is led to conclude that the proteins must be either cell surface proteins or extracellular proteins, the latter of which are, presumably, mucus proteins. Binding to mucus proteins is irrelevant to dosimetry, since the intracellular concentration of the toxicant, not the extracellular concentration of the toxicant, is of primary concern.

With regard to the final comments made by the Panel in this Section, we have never asserted that the amount of CH_2O bound to DNA following an acute exposure is necessarily the same as that following a chronic exposure. We do not know the amount of CH_2O bound under chronic exposure conditions. However, we do claim that short-term exposure conditions, which do not cause massive changes in cell structure and morphology, should more nearly represent the chronic exposure situation at low concentrations of CH_2O, i.e., those to which humans are actually exposed.

3.5 Nonlinearity for Crosslinked DNA at Low Doses

3.5.1 Documentation of Nonlinearity for Low Dose Crosslinked DNA

No comment.

3.5.2 Alternative Explanations for Nonlinearity of Low Dose Crosslinked DNA

The Panel suggests that, because each experiment involved only three replicates, it is possible that the results were spurious. They suggest that additional experiments below 6 ppm would add considerable substance to the results. We are surprised by this comment, since in our meeting with the Panel on December 2, we did present such evidence to them. As we showed at that meeting, we have carried out additional studies at 0.9, 2, 4, and 6 ppm (three replicates at each concentration) (Casanova-Schmitz and Heck, 1985). The results were fully consistent with those published previously.

The Panel also suggests the possibility of a "small but constant loss in the measurement of IF DNA" as being responsible for low-dose nonlinearity. In section 3.2, we showed that the Panel's earlier hypothesis of a dose-dependent loss of DNA was inconsistent with our results. The hypothesis of a dose-independent loss of DNA from the IF DNA fraction can be readily shown to be invalid. If one assumes a constant loss of IF DNA at all CH_2O concentrations, as suggested by the Panel, calculations can be made of the amount of IF DNA that would have to be lost at 2 and at 6 ppm in order to linearize the covalent binding curve. Such calculations were performed by us: the curve for covalent binding of CH_2O to DNA at 2 and at 6 ppm was recalculated using the equations in Appendix 3 of Casanova-Schmitz et al. (1984), but allowing for a constant loss of IF DNA. This loss would affect the binding calculation only by changing the fraction of DNA that is IF:

$$\text{Measured } (\%\text{IF DNA})/100 = (\text{IF DNA})/(\text{AQ DNA} + \text{IF DNA});$$

$$\text{"True" } (\%\text{IF DNA})/100 = (\text{IF DNA} + \underline{\text{Loss}})/(\text{AQ DNA} + \text{IF DNA} + \underline{\text{Loss}}).$$

Figure 2 shows the effect of loss on the ratio of the amount of covalent binding at 6 ppm to that at 2 ppm. While the ratio is reduced somewhat as the amount of lost IF DNA increases, in agreement with the suggestion of the Panel, it cannot drop below 8.2 even with arbitrarily large losses. However, in order to linearize the binding response, this ratio would have to drop to 3, the ratio of the two airborne formaldehyde concentrations. Therefore, a constant loss of IF DNA, no matter how large, cannot be responsible for the observed nonlinearity in CH_2O binding to DNA.

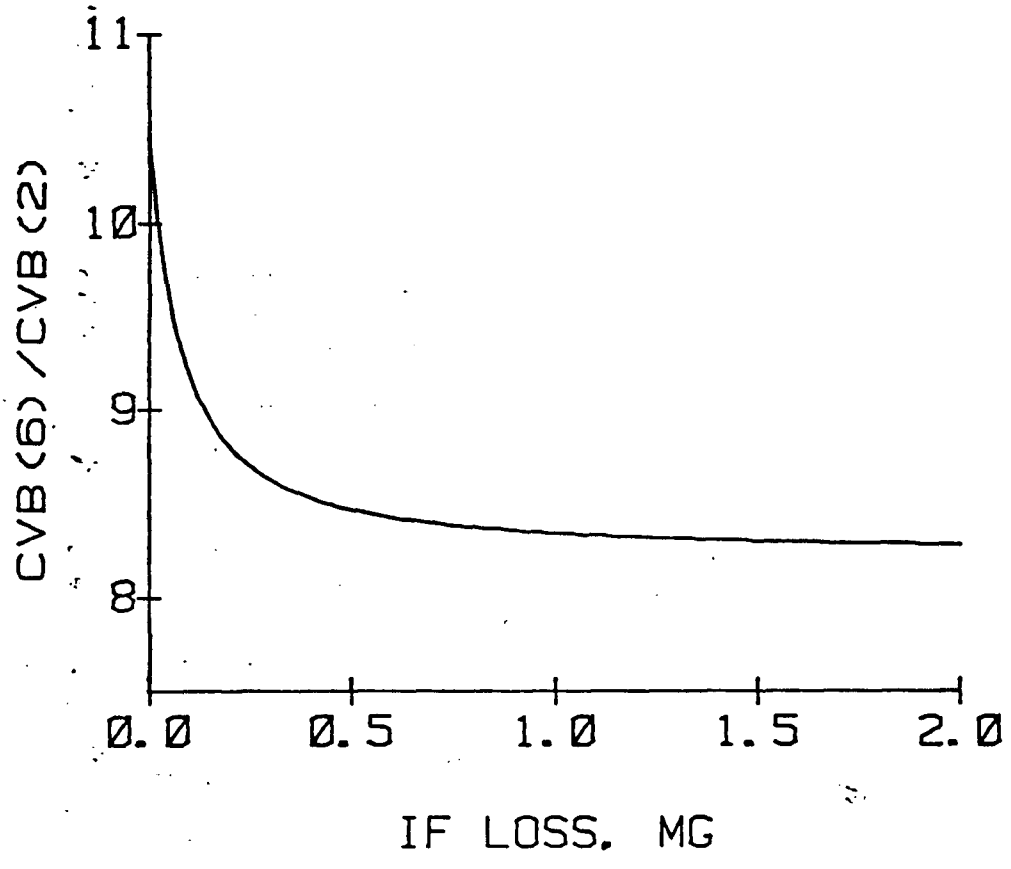


Fig. 2. Effect of a hypothetical constant loss of IF DNA on the calculated ratio of covalently bound CH_2O at 6 ppm and at 2 ppm. For linearity of the concentration-response curve, this ratio should be 3, the ratio of the two airborne CH_2O concentrations.

The Panel suggests that "the disproportionate increase in $^{3}H/^{14}C$ ratio with increase in CH_2O concentration might be due to artifactual disturbances ...rather than a true increase in crosslinked DNA-protein". What is meant by such "artifactual disturbances" is not clear, although the Panel appears to be referring to increases in cell turnover. We have already remarked that increases in cell turnover could well contribute to the low-dose nonlinearity in CH_2O binding to DNA (Casanova-Schmitz et al., 1984), just as saturation of metabolic defense mechanisms could also be a contributing factor. The point is that a change in the cell turnover rate or a change in CH_2O metabolism would result in a change in the amount of label metabolically incorporated into DNA. However, the AQ DNA provides a control for such changes, since the labeling of AQ DNA is only due to metabolism. It is the difference between the $^{3}H/^{14}C$ ratios of IF and AQ DNA, not the absolute value of the $^{3}H/^{14}C$ ratio of the IF DNA, that is directly related to the amount of covalently bound CH_2O.

Thus, the comment of the Panel does not call into question the validity of our conclusion that the increase in the $^{3}H/^{14}C$ ratio of the IF DNA relative to that of the AQ DNA is due to covalent binding. Rather, it simply suggests a mechanism for the disproportionate increase in covalent binding at high concentrations, i.e., increased cell turnover, which has already been proposed by us.

The last comments of the Panel in this section concern the possibility of cell death, which could (according to the Panel), "inhibit de novo nucleotide synthesis..., as well as increase the deoxynucleotide pools, thus, diluting

out radioactivity incorporated into DNA". The Panel then states that a "decrease in DNA synthesis...would tend to increase the $^{3}H/^{14}C$ ratio disproportionately at higher CH_2O concentrations". We repeat that any change in the labeling of DNA due to metabolism is already accounted for by our measurement of the radioactivity in the AQ DNA, which is only due to metabolism and is, therefore, an internal control for all such effects. Thus, the Panel has merely suggested a possible mechanism for low-dose nonlinearity. We doubt that this mechanism is plausible, however, because at low CH_2O concentrations (0.3, 2, and 6 ppm) where the nonlinearity occurs, cell death is not an important consideration. We know from the work of Dr. Kevin Morgan at CIIT that nasal mucociliary activity continues even after many days of exposure to 6 ppm of CH_2O.

3.6 Sensitivity of the Study Conclusions to Statistical Analysis

This section deals only with our statistical analyses of labeling, i.e., the ^{14}C-specific activity and the normalized $^{3}H/^{14}C$ isotope ratios in the IF and AQ DNA. The two-way analyses of variance of these measurements reported in Casanova-Schmitz et al. (1984) did not account for pairing between the IF and AQ DNA determinations or possible inhomogeneity of variance as a function of the exposure concentration. Furthermore, we did not specify ordered (over concentration) alternatives to the null hypothesis of no treatment effects prior to our examination of the data. Consequently, as noted by the Panel, these analyses had somewhat less than optimal power to detect systematic differences in treatment effects. Despite this limitation, statistically significant effects of exposure concentration and DNA fraction (including interac-

tion between these two factors) were detected in both the ^{14}C specific activity and the normalized $^{3}H/^{14}C$ isotope ratios. As has been verified subsequently, more sensitive statistical analysis procedures do no more than confirm these findings. Thus, as was noted by the Panel, our overall conclusions regarding these measurements are valid.

In the second and third paragraphs of this section, the ^{14}C specific activity measurements are described by the Panel as "bound (^{14}C) CH_2O". This confusing terminology can easily give readers the mistaken impression that covalent binding to DNA, rather than total ^{14}C specific activity in the two DNA fractions, is being discussed. We therefore strongly suggest changing this phrase to "^{14}C specific activity" or "total ^{14}C specific activity".

The absence of a statistically significant difference between the ^{14}C specific activities of the IF and AQ DNA fractions at 0.3 and 2 ppm is described by the Panel as "limiting the extent to which inferences can be made about the responses to low concentrations for purposes of identifying no-response levels or making low-dose extrapolations for risk assessment." We disagree with this statement. Such extrapolations should not be based solely on the difference between the ^{14}C specific activities of IF and AQ DNA, when it is the difference between the $^{3}H/^{14}C$ ratios (<u>not</u> the ^{14}C specific activity difference) that is the primary indicator of covalent binding to DNA. Significant differences between the $^{3}H/^{14}C$ ratios of the two DNA fractions were observed at all concentrations equal to or greater than 2 ppm. Consequently, the concentration of CH_2O covalently bound to DNA was found to differ significantly from zero at these concentrations as well.

3.7 Adequacy of the Measure of Exposure

No comment.

3.8 Utility of the Study in the Quantitative Risk Assessment of CH_2O

The Panel concludes that our results should not be used for risk assessment. Their conclusion is based on their arguments that: (1) experimental methodologies have not been properly "validated"; (2) intracellular proteins rather than DNA should be used as the target; and (3) acute exposures may not be relevant to chronic exposures. We disagree with the Panel on all points.

First, the experimental methodologies have been validated, both by HPLC analysis and by repetition of the experiments, as discussed above in Sections 3.1.1 and 3.5.2. We demonstrated the lack of variation of the DNA yield with concentration in Section 3.1.2, and we have determined the magnitude of the isotope effect in CH_2O oxidation and discussed its implications in Section 3.2 (the binding curve becomes more nonlinear rather than less). We showed in Section 3.5.2 that the assumption of a constant loss of DNA from the IF DNA fraction cannot account for low-dose nonlinearity no matter how large the loss is assumed to be. Finally, we discussed the fact that all experiments were carefully controlled to maintain constant extraction conditions for DNA at all exposure concentrations in Section 3.2.

Second, the argument that intracellular proteins should be used for dosimetry fails to recognize: (1) that covalent binding to proteins is not widely

acepted as a mechanism of mutagenesis, (2) that proteins have a much higher rate of turnover than DNA, and that individual proteins vary in their turnover rate, (3) that correcting for labeling of the proteins due to metabolism presents major experimental problems, and (4) that, in any case, protein binding occurs primarily on the extracellular proteins, not on the intracellular proteins. These points are discussed in detail in Section 3.4.

Third, the rationale for using acute exposure data for risk assessment at low concentrations is presented in section 3.2. These exposure conditions do not cause massive toxicity to the tissue, and, therefore, more closely represent the actual human chronic exposure situation. The results obtained provide information about the ability of CH_2O to react with DNA under realistic, low-level exposure conditions.

In summary, we find the concerns of the Panel to be without merit and their conclusions to be unsubstantiated.

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APPENDIX 2: INDIVIDUAL SUMMARIES OF EPIDEMIOLOGIC STUDIES REVIEWED

1. Matanoski (1982) of Johns Hopkins University examined mortality patterns of male pathologists in 2 professional societies, American Associations of Pathologists and Bacteriologists (AAPB) and the American Society for Experimental Pathology (ASEP). The results of these analyses were reported in a March 30, 1982 letter to John Martonik, Deputy Director of the Occupational Safety and Health Administration (OSHA). In separate analyses of each group, Matanoski compared the causes of death to the number of expected deaths using U.S. white males and using psychiatrists as the referent group. Additionally, Matanoski combined the two pathologist groups without overlap and compared proportions of deaths to those expected proportions using 1) psychiatrists and 2) internists, otolaryngologists, and opthalmologists as the referent.

In the SMR analysis, Matanoski observed the same pattern of deaths when either referent group was used as the standard. The present review has focused on results from comparisons with psychiatrists. For ASEP members, apparent excesses were observed for neoplasms of the liver (SMR=399, 3 observed), pancreas (SMR=277, 5 observed), and lymphomas (SMR=272, 3 observed). Deficits were observed for all deaths, all neoplastic deaths, and neoplasms of the lung, kidney, and bladder, brain, and lymphopoietic system. None were significant. For AAPB members, Matanoski observed

elevated mortality (not statiscally significant) from esophageal and small intestinal neoplasms (SMR=156, 2 observed), pancreas (SMR=263, 9 observed), brain (SMR=296, 5 observed), and lymphoma/multiple myeloma (SMR=174, 3 observed). Deficits were seen for all causes of deaths, all cancer deaths, and neoplasms of the stomach, large intestine, prostate, and lymphopoietic system. These deficits were not statistically significant.

Findings from the PMR analysis support the above observations. Matanoski, in addition, observed a statistically significant increase in the proportions of deaths due to neoplasms of the hypopharynx (PMR = 3060, $p \leq 0.005$, 2 observed).

2. Harrington and Shannon (1975) of the London School of Hygiene and Tropical Medicine conducted a SMR analysis of 2,079 pathologists who were members of the Royal College of Pathologists or the Pathological Society of Great Britain during the period of 1955 to 1973. By the end of 1973, 156 deaths occurred. The authors reported a significant excess in mortality from lymphopoietic system cancers (SMR=200, 8 observed, $p<0.05$), particularly from lymphatic and hematopoietic diseases not due to Hodgkin's disease or leukemia (SMR=353, 6 observed, $p<0.01$). Additionally, increased mortality from Hodgkin's disease appeared (SMR=167, 1 observed) for male pathologists in England and Wales.

Harrington and Shannon also presented an analysis of 12,944 laboratory technicians who were registered with the Council for Professions Supplementary to Medicine. Between August, 1963 and December 31, 1973, 154 deaths occurred. Deficits of deaths were observed from all causes and all neoplastic causes, including neoplasms of the digestive tract and peritoneum, lung, lymphohematopoietic system, Hodgkin's disease, and leukemia. These observations were not statistically significant. The most striking statistically significant excess in mortality was observed from suicides (SMR=243, 17 observed, $p<0.001$). In addition, Harrington and Shannon observed some excess in lymphohematopoietic deaths (not including leukemia or Hodgkin's disease) (SMR=118, 2 observed). This observation was not statistically significant.

3. Harrington and Oakes (1982) more recently followed the Royal College of Pathologists' cohort from 1974 to 1980 and performed an SMR analysis of 2,720 members (2,307 males and 413 females) in which 126 total deaths (110 males, 16 females) occurred. Harrington and Oakes observed increased mortality in males from cancers of the brain (SMR=331, 4 observed, $p<0.05$) and bladder (SMR=107, 2 observed), from accidents (SMR=170, 13 observed $p<0.05$), and from suicides (SMR=353, 7 observed $p<0.01$). Increased mortality from lymphatic and hematopoietic neoplasms was not reported for male

pathologists (2 observed deaths) but was reported for female pathologists (SMR=370, 1 observed death). Deficits in mortality (statistically significant, $p<0.05$) were observed for males from all neoplasms (SMR=61, 32 observed) and from neoplasms of the lung (SMR=41), 9 observed) and digestive and peritoneal system (SMR=51, 8 observed). All the malignant brain tumors diagnosed in males were of the astrocytoma/glioma cell type.

4. Levine et al. (1984) of CIIT in an SMR analysis found excess mortality among Ontario morticians, relative to Ontario white males, from lymphopoietic cancer (SMR=124, 8 observed), particularly, leukemias/aleukemias (SMR=160, 4 observed), and brain cancers (SMR=115, 3 observed). None of these malignancies was significantly elevated. Only cirrhosis of the liver and rheumatic heart disease (SMR=199, 8 observed, $p<0.05$) showed significant excesses (SMR=171, 18 observed, $p<0.001$). Decreases in mortality (not statistically significant) were observed for neoplasms of the lung, digestive system, and buccal cavity.

In an earlier analysis of these deaths where U.S. white males were used as the standard population, Levine observed increasing SMR's with increasing time since first exposure for cancers of the brain, lymphopoietic system, and leukemia/aleukemia.

Levine et al. did not report exposure levels to which Ontario undertakers may be exposed, but data from a survey of seven West Virginia funeral homes were presented. Mean time-weighted averages for breathing zone samplers showed HCHO levels between 0.3 ppm and 0.9 ppm (Williams et al., 1984). It is not known how similiar the exposures are between these 2 groups.

5. Stroup (1984) noted excesses, when compared to U.S. white males, in mortality due to brain cancers (SMR=271, 10 observed, $p<0.01$) and leukemias (SMR=148, 10 observed) in her unpublished SMR study of 2,239 anatomists who were members of the American Association of Anatomists (AAA). Stroup noted excesses of the cell types astrocytoma/glioblastoma (all ten brain tumors) and myeloid leukemia (5 of the 10 leukemia deaths). Deficits in mortality were observed for neoplasms of the lung (SMR=28, 12 observed, $p<0.05$), buccal cavity and pharynx (SMR - 15, 1 observed, $p<0.05$) and of the nasal cavity or sinuses (0 observed, 0.14 expected).

Stroup further examined the relationship between exposure and mortality from brain cancer and leukemia. The number of years of membership in the AAA was used as a surrogate for exposure. In these analyses, only the SMR's for brain cancer increased as the membership years increased. To further examine the relationship between mortality from the above three neoplasms and exposure to formaldehyde, Stroup categorized the anatomists'

subspecialities by their potential usage of HCHO. Gross anatomists were classified as having high HCHO exposure, anatomists who had specialities in both gross anatomy and microanatomy had medium exposure, and microanatomists had low HCHO exposure. No trend with formaldehyde rank was observed. This analysis, however, may be limited by the small numbers of deaths (less than 5 in each category) and may be biased due to exposure misclassification.

To examine if social class differences or if ascertainment may have biased the observed excess brain cancer and leukemia mortality, Stroup used psychiatrists as a comparison group. In this comparison, the excess brain cancer and leukemia mortality remained (brain cancer, SMR=572, $p<0.01$; leukemia, SMR=212, $p<0.05$). It can be concluded from these analyses that neither social class differences nor ascertainment bias accounts for the observed increased in brain cancer and leukemia mortality.

6. Wong (1983) observed that among 2,026 workers employed by Celanese in a HCHO manufacturing plant, as compared to U.S. males, mortality was increased (not statistically significant) from neoplasms of the skin (SMR=109, 95% CI*:2-717, 1 observed), bone (SMR=430, 95% CI:6-2751, 1 observed), prostate (SMR=305, 95% CI:84-797, 4 observed), bladder (SMR=122, 95% CI:2-705,

* 95% Confidence Interval (95% CI)

1 observed), kidney (SMR=102, 95% CI:1-613, 1 observed), brain (SMR=186, 95% CI:43-623, 3 observed), and lymphopoietic system (SMR=136, 95% CI:43-623, 3 observed), and lymphopoietic system (SMR=136, 95% CI:57-338, 6 observed), including Hodgkin's disease (SMR=240, 95% CI:33-1063, 2 observed) and leukemia/aleukemia (SMR=118, 95% CI:15-487, 2 observed). Lung cancer mortality was decreased (SMR=82, 95% CI:37-156, 9 observed) and no nasal cavity or sinus neoplastic deaths were observed. Accounting for a latency of 20 years, Wong observed significantly increased mortality from cancer of the prostate (SMR=431, 4 observed, $p<0.05$) and apparently increased mortality from lymphopoietic system (SMRS=231, 95% CI:62-591, 4 observed), including Hodgkin's disease (SMR=582, 95% CI:8-3236, 1 observed). Again, lung cancer mortality was decreased (SMR=87, 95% CI:32-190, 6 observed). Besides HCHO, this cohort had potential exposures to other oxygenated hydrocarbons, benzene, asbestos, and inorganic and organic pigments. Exposure to benzene is particularly important since the literature reports a causal association between leukemia and benzene exposures (Heath, 1982).

7. Tabershaw Associates (1982) studied the same cohort as Wong, with 58 men added who had incorrectly been excluded and with the HCHO-exposed workers identified. An SMR analysis of the exposed and unexposed cohorts and a case-control analysis using randomly-selected controls

among the non-cancer cases were conducted. In the SMR analysis of 867 HCHO-exposed workers, increased mortality (not statistically significant) from prostatic (SMR=364, 2 observed), brain/CNS (SMR=135, 1 observed) cancers and from all accidents (SMR=103, 11 observed) was reported. Interestingly, Tabershaw Associates base the brain/CNS conclusion on one observed death, yet the text indicates that 2 men who had 6.7 years and 24 years of exposure died of this cause. Decreased mortality from lung cancer (SMR=54, 3 observed) was observed along with no deaths from neoplasms of the digestive organs and peritoneum, nasal cavity and sinuses, and bladder.

In the case-control analysis, increased odds ratios for cancers of the prostate (OR=2.67) and lymphopoietic system (OR=3.0), and for all neoplasms (OR=1.2) were reported for cases with 5 to 15 years of HCHO exposure. These increases were not statistically significant. Risks did not appear to increase with increasing years of exposure or increasing number of years employed at the plant. Note that Tabershaw Associates did not use an unexposed group as a comparison, but compared the exposed employees to those with less than 5 years of exposure. The use of this group as the "controls" may diminish the ability of this analyses to detect a small elevation in risk due to a higher background prevalance of formaldehyde exposure.

8. Acheson (1984a) of MRC Environmental Epidemiology Unit, Southampton General Hospital, in an ongoing study of 7,716 workers in six plants which use or manufacture HCHO, has observed significant decreases in overall mortality (SMR=87, 1,619 observed, $p<0.05$) and nonsignificant increases from buccal cavity and pharyngeal (SMR=121, 5 observed), esophageal (SMR=103, 13 observed), respiratory (SMR=102, 236 observed), and lung (SMR=105, 205 observed) cancers.

Acheson et al. subjectively categorized exposure on the basis of workers' recall of acute irritation. These categories were defined as: nil/background, <0.1 ppm; low, 0.1-0.5 ppm; moderate, 0.6-2.0 ppm; high, >2.0 ppm. In analyses based on these categories, Acheson et al. found a significant excess of bone cancer mortality and a significant dose-response relationship for lung cancer mortality in one plant (BIP), the cohort with the highest exposure. In a comparison with local controls, the dose-response relationship (marginally significant) was still observed. Acheson lacked smoking histories for the entire cohort, and the BIP plant is located in the West Midlands area, an industrially polluted area with high referent lung cancer rates. The use of a local comparison may have overestimated the number of expected lung cancer deaths (Enterline, 1976) since the local lung cancer rates may be influenced by the BIP lung cancer deaths; reducing the power of the analysis.

In subsequent analysis of the mortality data for lung cancer among individuals employed at this plant, Acheson et al. (1984b) observed that the risks for lung cancer did not increase with duration of employment or with cumulative doses (as assessed by three measures).

In a third analysis of all 120 lung cancer deaths and 640 controls in the BIP plant, Acheson et al. (1984c) examined smoking history and previous employment. Acheson et al. found no differences between the cases and their controls. Only 11% of the cases and 12% of the controls had adequate information on smoking, however.

9. Marsh (1983a) of the University of Pittsburgh conducted an SMR analysis and a case-control study nested within the cohort study of Monsanto chemical workers. This plant produced plastics and workers had potential exposures to HCHO, vinyl chloride, styrene, and cellulose acetate. Marsh compared the mortality experience of all workers to the white male populations of the U.S., of Massachusetts, and of Hampden County, the county from which the workforce was drawn. In the SMR study, the cohort consisted of 2,490 male workers with a minimum of one year employment. Among the 2,490 workers, 591 deaths were identified by the company or by death certificate searches. Marsh reported increased mortality (not statistically significant) due to all neoplasms (SMR=107, 127 observed). Among all neoplasms,

excess mortality was observed from cancer of the buccal cavity and pharynx (SMR=155, 6 observed), digestive organs and peritoneum (SMR=126, 44 observed), prostate (SMR=178, 14 observed), bladder (SMR=135, 5 observed), genitourinary tract (SMR=169, 26 observed, $p<0.05$), Hodgkin's disease (SMR=118, 2 observed), and all other lymphopoietic tissue (SMR=153, 4 observed). No relationship was observed between genitourinary system neoplasms and length of employment.

In the matched case-control study based on the cancer deaths, Marsh presented odds ratios for digestive system, rectal, genitourinary, and prostatic cancers and 21 occupational exposure categories. Two of the 21 categories had pertinent exposure to HCHO either as a chemical (resin production) or in a product (resins processing). Marsh observed increased odds ratios for digestive system cancer in the resins processing category (OR=1.83) and for rectal cancer in both categories (resins production, OR=3.75; resins processing, OR=2.00). These increases were not statistically significant. All cases in the occupational categories had from 1 month to 5 years exposure and increasing risk was not observed with increasing duration of exposure.

10. Bertazzi et al. (1984) of the Institute of Occupational Health, University of Milan, presented at the 3rd International Conference on Epidemiology and

Occupational Health findings of a cohort study of HCHO resin manufacturing workers. The mortality experience of 1,332 male employees who had worked six (6) months or more between 1959 and 1980 was compared to the expected number of deaths using national and local rates. Bertazzi et al. noted that ambient monitoring showed many work areas were above the Threshold Limit Value. Area sampling values between 1974 and 1979 ranged between 0.2 and 3.8 mg/m^3.

For the entire cohort, Bertazzi et al. observed significantly increased mortality for all neoplasms (SMR = 154, 42 observed, $p<0.05$ national rates) and for lung cancer when both national (SMR = 236, $p<0.05$ 18 observed) and local (SMR = 186, $p < 0.05$) rates were used as the referent. Mortality from digestive (SMR = 156, 14 observed, national rates) and lymphopoietic (SMR = 201, 5 observed, national rates) and esophageal-stomach (SMR = 148, 70 observed, national rates) neoplasms were apparently elevated.

Bertazzi et al. had work histories for all but 18 percent of the cohort. Using local rates as the standard, Bertazzi et al. examined mortality among formaldehyde-exposed workers. In this analysis, mortality appeared elevated from all causes (SMR = 111, 51 observed), and all neoplasms (SMR = 128, 19 observed), particularly alimentary tract (SMR = 155, 8 observed), lung (SMR = 136, 5 observed) and

hematologic (SMR = 273, 3 observed) neoplasms. In analyses examining cause-specific death by duration of exposure, only the SMR's for lung cancer increased as the number of years employed increased. Statistical testing was not performed to see if this trend was significant.

11. Blair et al. (1986) of the National Cancer Institute and the Formaldehyde Institute conducted a SMR study of 26,561 workers in 10 plants which manufacture or use formaldehyde; 7 plants produced resins, 2 plants photographic films, and one plant produced plywood. Two of the 10 plants were included in the studies of Wong (1983), Tabershaw Associates (1982), Marsh (1983a and 1983b), and Liebling et al. (1984). The Blair et al. study cohort was the largest ever studied for formaldehyde exposure. Any worker who had ever been employed in any one of the 10 plants was included in the cohort. Over 80 percent of the cohort were white males.

Blair et al. examined the relationship between mortality and exposure as categorized 3 ways: 1) time-weighted average (TWA), 2) peak exposure, and 3) cumulative exposure in ppm-years.

In the analysis of TWA exposure, Blair et al. examined the mortality experiences of white males with TWA exposures of >0.1 ppm (exposed) and compared their experience to the mortality experience of white males with TWA exposure of $\leq$0.1 ppm (nonexposed). Elevations

in the SMR over 100 for the exposed appeared present for cancer of the prostate (SMR=115, 33 observed), liver (SMR=102, 11 observed), lung (SMR=111, 201 observed), bone (SMR=123, 40 observed) and kidney (SMR=123, 18 observed) and for Hodgkin's disease (SMR=142, 14 observed, while the SMR's of the nonexposed were less than 100. None of the elevated SMR's was statistically significant. In the analysis of peak exposure, only Hodgkin's disease among white males showed a significant increasing trend with intensity of exposure. This trend also appeared present, but was not statistically significant, when cumulative exposure was examined.

In analyses examining cumulative exposure, Blair et al. observed among white males a significant elevation of neoplasms of the lung (SMR=122, 212 observed, $p \leq 0.05$) and nasopharynx (SMR=300, 6 observed, $p \leq 0.05$) whose cumulative exposure to formaldehyde was greater than 0 ppm-years. A trend with increasing exposure was not reported for either site. When latency, defined as >20 years, was accounted for, the lung cancer excess increased and remained significant (SMR=135, 146 observed, $p<0.05$), but the nasopharyngeal excess became marginally significant (SMR=300, 3 observed, $p = 0.08$). Again, no trend with increasing exposure was observed for either site.

Exposure characterization for early exposures may be subject to recall and misclassification bias,

although an elaborate exposure matrix was developed for this study. Historic exposures were estimated from sensory perception, previous monitoring, and current levels with knowledge of plant process changes. Current levels were determined using three methods, NIOSH P&CAM 125 area monitoring, and DuPont and 3M passive dosimeters. The use of passive monitors for low level, short term exposures may not be valid and their use, even weighted, may not be appropriate. Second, formaldehyde exposure levels for several jobs were below the analytical method's level of detection, with the job identified as having an exposure of 0.0 ppm. Thus, weighting the exposures associated with 0.0 ppm (i.e., below the analytical method's level of detection) may be inappropriate. Third, industrial hygiene data show wide variation in formaldehyde levels for a single given job. Fourth, sensory perception to formaldehyde may be influenced by recall. All of these factors compound to reduce the certainty of the exposure categorization.

Blair et al. argue that this study provides little support that formaldehyde exposure is associated with cancer. The authors based this conclusion on the lack of a dose-response relationship between exposure and lung and nasopharyngeal neoplastic mortality. Apparent lack of an exposure-dose trend cannot diminish the importance of the 30% excess in lung cancer mortality and the 200% excess in nasopharyngeal mortality, for

these are statistically significant increases. Smoking may not account for the observed excesses in lung cancer mortality; when the SMR's across exposure groups for lung cancer are compared with another smoking-related endpoint, emphysema, the SMR's for emphysema decrease with increasing exposure whereas the SMR's for lung cancer remain elevated.

There may be several reasons for the observed lack of a dose-response trend in this study. Most importantly, misclassification and recall bias may be present. Second, even though workers who began employment in the 1930's and 1940's are included in this cohort, 44% of the cohort entered the study between 1956 and 1965. Since vital status was obtained in 1980, a full latency period for these workers may not have been obtained and may bias the results towards the null hypothesis of no effect.

Subsequent to the release of this study, OTS/EPA has received notice that 4 of the 6 nasopharyngeal cancer deaths occurred at 1 plant which manufactured resins and molded compounds. There is a significantly elevated SMR for this plant (SMR=920, 4 observed, $p<0.01$). All 4 workers died 15 or more years since first exposure and all 4 deaths had worked in the early part of their employment in the same position. The only other pertinent exposure besides formaldehyde was to cellulose pulp dust (personal conversation with Dr. Jim.

Collins, Manager of Epidemiology, American Cyanamid Company).

Blair et al. (1987) performed analyses of the nasopharyngeal and oropharyngeal cancer deaths which examined particulate exposure. In these analyses, Blair et al. observed for those workers with particulate exposure an apparent increasing trend between nasopharyngeal cancer mortality and cumulative formaldehyde exposure, however, this trend was not statistically significant at a $p=0.05$ level. No trend was seen between nasopharyngeal cancer mortality and cumulative formaldehyde for those workers not exposed to particulates nor was there a trend for oropharyngeal cancer by cumulative formaldehyde, regardless of particulate-exposure status. These analyses are limited by the small number of deaths in the subcategories. In addition, it is possible that the nasopharyngeal cancer-formaldehyde dose-gradient, is a surrogate for an unmeasured particulate gradient. Blair et al. (1987) believe, however, that formaldehyde and particulates together appear to be a risk factor for nasopharyngeal cancer. Blair et al. (1987) postulated that the delivered formaldehyde dose for these workers may be higher than was estimated in Blair et al. (1986) due to formaldehyde attachment to the particulate matter.

12. Stayner et al. (1986) of the National Institute of Occupational Safety and Health (NIOSH) conducted a

cohort mortality study of 11,030 workers in 3 garment facilities that used formaldehyde resins in the production of permanent press garments. Two of the three facilities were included in the proportionate mortality analyses of Stayner et al. (1985). In this analysis, which is described in #17, only the proportions of deaths in 3 plants were analyzed. In the present cohort study, 1 plant in the PMR was replaced with another larger plant, in terms of the number of employees, from another company.

Garment workers included in the study cohort must have worked for at least 3 months between the time when formaldehyde fabrics were first introduced into the production process and December 31, 1977. The cohort was composed mainly of workers who were white women, were from plant 1, had been employed from 3 months to 4 years, and had first exposures before 1963.

Free formaldehyde was extensively measured by NIOSH investigators between 1981 and 1984 using both area samplers and personal monitors. The time-weighted-average HCHO level for these years was 0.15 ppm (a range of 0.14-0.17). Stayner et al. sampled for potential confounding exposures such as phenol, organic solvents, and dust. These industrial hygiene surveys did not identify any chemical exposures which could result in substantial confounding. Likewise, nuisance dust levels were minimal.

A total of 609 deaths (822 expected; SMR=74) were observed among 188,025 person-years. No significant deficits in site-specific deaths were noted by the authors. Stayner et al. observed statistically elevated excesses in deaths due to cancer of the buccal cavity (4 observed, SMR=343, $p<0.05$), tonsils (2 observed, SMR=694, $p<0.05$), and connective tissue (4 observed, SMR=364, $p<0.05$). The statistical test employed by Stayner et al. is one-sided since the investigators had *a priori* planned to examine the relationship between formaldehyde exposure and elevations in cancer mortality (not whether formaldehyde exposure was related to change in cancer mortality, use of a two-sided test statistic). All 4 of the buccal cavity deaths were among females whose first exposure was between 1955 and 1962. Two of the 4 buccal cavity cancer deaths were parotid tumors, and the other 2 deaths were cancers of the oral mucosa and soft palate.

There appeared to be an increase in mortality from neoplasms of the lung, trachea, and bronchus (39 observed, SMR=114) and other lymphopoietic sites (5 observed, SMR=170), from leukemia (9 observed, SMR=114), and from bronchitis (SMR=190, n=4). Mortality from neoplasms of the brain appeared decreased (5 observed, SMR=71). No deaths from nasal cancer were observed in this cohort.

Stayner et al. analyzed the data by plant, by length of latency, and by duration of exposure. In these analyses, Stayner et al. observed elevated mortality from cancers of the trachea, bronchus, and lung (29 observed, SMR=149, $p<0.05$) and connective tissue (3 observed, SMR=514, $p<0.05$) in plant 1 only. Stayner et al. noted that the remaining 2 plants each lacked sufficient power to detect small to moderate elevations in lung cancer risks. In the analyses which examined duration of exposure and latency period, Stayner et al. observed the highest excesses in mortality from cancers of the buccal cavity, connective tissue, and other lymphopoietic tissue among those workers with the longest duration of exposure (10+ years) and with the greatest latency period (20+ years). In these analyses, the risk of lung cancer appeared to decrease with increasing duration of exposure. An EPA analysis of the data in the paper shows there is a statistically significant increasing trend ($p < 0.05$) between buccal cavity cancer mortality and duration of exposure, although such an analysis had not been performed by Stayner et al.

Stayner et al. concluded that the excesses in mortality from buccal cavity neoplasms, leukemia, and other lymphopoietic neoplasms were consistant with the hypothesis of being formaldehyde related. It is not known how the excess in connective tissue cancer

mortality may relate to exposure. It must be recognized that these findings are based on a small number of cases and that confounding with other exposures may exist. The investigators believe, however, indirect evidence suggests that cigarette and alcohol consumption were not confounders for the observed excess in buccal cavity cancer mortality.

13. Walrath and Fraumeni (1983) of NCI conducted a PMR study of 1,132 funeral directors or embalmers licensed in New York. In this cohort, Walrath and Fraumeni observed significantly elevated mortality from skin (PMR=221, 8 observed, $p<0.05$) and colon (PMR=143, 29 observed, $p<0.05$) neoplasms. Elevations also appeared present for cancer of the buccal cavity and pharynx (PMR=113, 8 observed), digestive system (PMR=104, 68 observed), liver (PMR=106, 5 observed), pancreas (SMR=105, 13 observed), lung (PMR=108, 72 observed), brain/CNS (PMR=156, 9 observed), kidney (PMR=150, 8 observed), and lymphatic/hematopoietic system (PMR=121, 25 observed). No nasal cavity and sinus neoplasms were observed. Among those licensed as embalmers only, Walrath and Fraumeni observed increases in mortality from buccal cavity and pharyngeal (PMR=201, 7 observed), skin (PMR=326, 5 observed, $p<0.05$), and brain/CNS (PMR=234, 6 observed, $p<0.05$) cancers. In the analysis for latency, Walrath and Fraumeni observed, for the entire

cohort, increasing PMR's for skin ($p<0.05$) and brain/CNS neoplasms with increasing time since first licensed.

Walrath and Fraumeni did not report actual exposure data for these embalmers, but data from previous industrial hygiene surveys were presented. In a NIOSH survey of a mortuary science college, formaldehyde levels ranged between 0.2 and 0.9 ppm. Another survey of 6 funeral homes reported formaldehyde levels ranging from 0.1 to 5.3 ppm.

14. Walrath and Fraumemi (1984) conducted another PMR analysis of 1,050 embalmers in California and observed similar findings as from the analyses of N.Y. embalmers. Walrath and Fraumeni reported significantly increased mortality from neoplasms of the brain (PMR=193, 9 observed, $p<0.05$), leukemia (PMR=175, 12 observed, $p<0.05$), and prostate (PMR=176, 23 observed, $p<0.05$). Increases that were not statistically significant were also reported for lymphatic and hematopoietic system (PMR=123, 19 observed) and buccal cavity and pharyngeal (PMR=131, 8 observed) cancers. Mortality from lung neoplasms was slightly decreased (SMR=96, 41 observed). As in the previous Walrath and Fraumeni study, no neoplasms of the nasal cavity and sinuses were reported.

Walrath and Fraumeni did not report actual exposure data for these embalmers, but the above-mentioned industrial hygiene data were included.

15. Marsh (1983b) of the University of Pittsburgh conducted a PMR analysis of HCHO-exposed workers at the Monsanto plant described previously. Marsh found 136 deaths among male workers with exposure of 1 month or greater in a "formaldehyde related plant area". Marsh compared their mortality experience to U.S. male, age-race adjusted, proportionality mortality data.

In the HCHO-exposed white males (115 deaths), Marsh observed increased (not statistically significant) mortality from cancers of the genitourinary system (PMR = 121, 3 observed), including the bladder (PMR = 330, 2 observed) and of the digestive organs and peritoneum (PMR = 127, 8 observed), particularly the pancreas (PMR = 160, 2 observed). Marsh observed decreased mortality from all neoplasms (PMR = 90, 20 observed), particularly the respiratory system (PMR = 80, 2 observed).

Only 21 deaths occurred among non-white formaldehyde-exposed males, and 2 of these deaths were from neoplasms (1 respiratory, 1 genitourinary). Marsh does not report PMR's for those sites where less than 2 deaths occurred.

In this study, Marsh additionally examined cause-specific mortality for those workers not exposed to formaldehyde. The most striking observations were of significant excesses in deaths from genitourinary tract neoplasms (white males), (PMR = 192.3, 22 observed,

$p<0.05$) and from all neoplasms (non-white males), (PMR = 251, 5 observed, $p<0.01$), particularly other malignant neoplasms (PMR = 882, 3 observed, $p<0.01$). Marsh does not present exposure information for the formaldehyde-exposed workers, but white male neoplastic, respiratory cancer, and genitourinary cancer deaths were analyzed by duration of employment (since being exposed to formaldehyde). Only the PMR's for respiratory cancer increased with increasing years of employment.

Since Marsh published this study, Infante of OSHA has found 1 cancer of the nasal sinus and 1 naso-pharyngeal cancer. Both men died 3 years after Marsh's follow-up period. The worker who died of cancer of the nasopharynx was a member of Marsh's cohort, but had been counted as living since he had not died at that time.

16. An overlapping study was conducted by Liebling et al. (1984). Liebling et al. identified 24 male workers who died between January 1, 1976 and December 31, 1980 through union records, reports of former co-workers, and a systematic review of obituaries in local newspapers. Work histories were obtained from seniority lists.

Proportionate mortality ratios were calculated to examine cause-specific mortality using the age, sex, race and cause-specific mortality proportions of the U.S. and county in which the plant is located. To adjust for the healthy worker effect, age, sex, and race-standardized PCMR's based on county comparisons

were also calculated. Deaths among 18 white and 6 black males with known HCHO exposure were identified. Race-age-sex adjusted PMR's were significantly elevated for cancer of the colon based on U.S., county, and county cancer mortality proportions (PMR = 702, 424, 333, $p \leq 0.05$), as were PMR's for buccal and pharyngeal cancer (PMR = 870, 952, 833, $p \leq 0.05$). Liebling et al. stated that the occurrence of a significant increase in proportionate mortality from buccal and pharyngeal cancer in this investigation is in accord with the type of cancer found in HCHO-exposed rodents. Furthermore, the authors postulated that besides nasopharyngeal cancer, an association between HCHO exposure and cancer of the buccal cavity and pharynx in humans is biologically feasible since humans breathe through both the nose and mouth, while rats and mice are obligatory nose-breathers. Like many other studies, this study is limited by the inability to completely separate HCHO exposure from exposure to other chemicals.

17. Stayner et al. (1985) of NIOSH conducted a PMR study of 256 deaths among garment workers in 3 plants. Two of these plants were included in the cohort study identified in #12 (Stayner, 1986). Stayner et al. identified the 256 deaths from a death benefit fund. In this cohort Stayner et al. observed significantly elevated mortality from buccal cavity (PMR=750, 3 observed, $p<0.001$), biliary passages and liver

(PMR=313, 4 observed, $p<0.01$), and other lymphatic and hematopoietic site (PMR=400, 4 observed, $p<0.05$) cancers. In analyses examining only the cancer deaths, buccal cavity (PCMR=682) and other lymphatic and hematopoietic site (PCMR=342) cancers remained significantly elevated ($p<0.01$ and $p<0.05$, respectively). Additionally, those workers with both latency and duration of exposure of 10 years or greater showed significantly elevated mortality from all malignancies (PMR=137, 51 observed, $p<0.05$), buccal cavity (PMR=925, 2 observed, $p<0.05$), biliary passages and liver (PMR=467, 3 observed, $p<0.05$), and all lymphatic/hematopoietic sites (PMR=283, 8 observed, $p<0.05$), particularly other lymphatic and hematopoietic (PMR=761, 4 observed, $p<0.05$) cancers.

Nonsignificant elevations in mortality were reported for liver not specified (PMR=426, 2 observed), skin (PMR=179, 2 observed), and all lymphatic and hematopoietic sites (PMR=163, 10 observed), including leukemia (PMR=400, 4 observed).

18. Delzell and Grufferman (1983) of Duke University examined the mortality experience of 4,462 deaths between 1976-1978 of white female textile workers. Deaths and occupation as recorded on the death certificates were identified from state computer files. In this study the textile worker occupational code included workers in industries that manufactured

textile mill products, apparel, or other fabricated textile products. Delzell and Grufferman observed significant excesses in mortality from cancer of the larynx (PMR = 280, 5 observed, $p<0.05$), connective tissue (PMR = 260, 10 observed, $p<0.05$), cervix (PMR = 210, 59 observed, $p<0.05$), other unspecified genital organs (PMR = 270, 16 observed, $p<0.05$), and all lymphopoietic sites (ICDA 200-207) (PMR = 130, 121 observed, $p<0.01$), particularly non-Hodgkin's lymphoma (PMR = 170, 51 observed, $p<0.05$). Decreases in mortality that were not statistically significant were observed for neoplasms of the lung (PMR = 90, 106 observed) and of the brain (PMR = 90, 17 observed).

This study is limited by the unknown exposure status of each death. The occupational code, textile worker, was used as an indirect measure of formaldehyde exposure. This study is unable to identify whether formaldehyde had actually been an exposure, and if so, in what concentrations. A second limitation is the insensitivity of death certificate occupational code analyses.

19. Fayerweather et al. (1982) of DuPont showed elevated odds ratios, after a 15 to 24 year latency, for cancers of the prostate (OR=4.8, 8 cases), lymphopoietic system (OR=1.91, 6 cases), bone (OR=1.25, 3 cases), and bladder (OR=7.0, 6 cases) among workers eligible for pension were exposed to HCHO 5 or more years. Decreases in

mortality were observed for those employees working 5 or more years from colorectal (OR=0.74, 8 cases) and lung (OR=0.79, 15 cases) neoplasms. No difference in mortality was observed for buccal cavity neoplasms (OR=1.0, 1 case).

Fayerweather et al. examined formaldehyde exposure by 3 ways: by the number of years worked around formaldehyde (less than 5 years, 5 or more years), whether the employee had intermittent or continuous formaldehyde exposure, and by a cumulative exposure index. In these analyses, only the odds of mortality from bladder and from prostatic cancer increased as the exposure indices increased.

Fayerweather et al. did not follow those employees ineligible for pension or those who had transferred, potentially comprising 15 to 20% of the work group.

20. Brinton et al. (1984a) of NCI conducted a case-control study for cancer of the nasal cavity and sinuses. They observed nonsignificantly increased odds ratios among males employed in the leather or shoe, chemical manufacturing, and carpentry industries and for exposures to chromium/chromates, nickel, and insecticides/pesticides/herbicides. Among females, increased odds ratios were observed with employment in the textile/clothing/hosiery and paper/pulp mill industries and for exposures to mineral oils and other mineral/chemical gases. None of the increased odds

ratios was significant in the presence of control for confounding variables. Brinton et al. additionally assessed reported occupational HCHO exposure and found an odds ratio less than 1.0. This ratio was unstable based on only one male and one female.

To examine the relationship between employment in the textile and apparel industries with the risk of nasal cancer, Brinton et al. (1984b) further analyzed the data from their previously published case-control study (1984a). The industries included textile and cotton mills, apparel manufacturing, and hosiery. Brinton et al. found an elevated risk of nasal cancers associated with employment in the textile or apparel industries, but the increased relative risk was found only among female workers. When histologic types of nasal cancer were evaluated, both males and females were found to be at increased risk of nasal adenocarcinoma, with further enhancement of risks with exposure to dusty work conditions. Few individuals in this analysis had exposure to formaldehyde (2 cases) and the ratio was below 1.0 (OR=0.4). The authors considered this study to provide further evidence of an association between employment in the textile industry and risk of nasal cancer. This study had limited ability to evaluate nasal cavity cancer and a direct assessment of formaldehyde exposure.

21. Tola et al. (1980) of the Institute of Occupational Health, Finland, conducted a case-control study for cancer of the nose and paranasal sinsuses. Forty-five cases were collected from the Finnish Cancer Registry between 1970 and 1973 and were age-sex matched to nonrespiratory cancer controls.

Analyses examining an occupational etiology showed no single occupation being more common among the cases than among the controls, but leisure time knitting and sewing was significantly more common among female cases than among female controls (OR=4.8, 19 cases). Other factors significantly associated with the cases were histories of serious nasal trauma, chronic rhinitis, and sinusitis. Smoking was not significantly associated with nasal cavity and sinus cancer.

This study is limited in its ability to evaluate formaldehyde exposure. Occupational histories were obtained for 69% of the cases from a next-of-kin, potentially biasing the study towards the null hypothesis of no-association.

22. Hernberg et al. (1983) of the Institute of Occupational Health, Finland, conducted, with participation from Denmark and Sweden, a collaborative case-control study of nasal and sinonasal cancer and its possible occupational etiology. One hundred seventy cases diagnosed between 1977 and 1980 and reported to the prospective cancer registries were selected. Each case

was sex-country-age at diagnosis matched with colorectal cancer controls.

Elevated odds ratios were observed among cabinet-makers (OR=9.0) and mechanical engineering shop workers (OR=2.13). Analysis for exposures showed elevated risk with hardwood dust (OR=1.7)*, softwood dust (OR=3.4, $p<0.05$)*, hardwood and softwood dust (OR=67, $p<0.05$)*, welding-flame cutting-soldering (OR=2.0, 17:6, $p<0.05$)**, chromium (OR=2.7, 16:6, $p<0.05$)**, nickel (OR=2.4, 12:5)** electroplating (OR=1.5, 9:6)**, and paint-lacquer (OR=3.0, 18 cases). HCHO exposures may occur in this last category. However, wood dust exposure is common and confounds the observed elevation.

23. Hardell et al. (1982) of Umea, Sweden conducted a case-control study of nasal and pharyngeal cancers. Seventy-one cases, first diagnosed between 1970-1979, and 541 referents were specifically studied for relationships with phenoxy acid or chlordane exposure. Cases were selected from the Swedish Cancer Register and referents were utilized from earlier case-control studies of soft tissue sarcoma and lymphoma and of colon neoplasms. These referents were apparently representative of the population between 1970 and 1978.

Hardell et al. observed increased risks with exposure to chlorophenol (OR=6.7, 95% CI:2.8-16.2) and

*Adjusted for smoking.
**Odds ratio based on discordant pairs, concordant: discordant pairs noted.

phenoxy acids (OR=2.1, 95% CI:0.9-4.7). The odds ratio for chlorophenol remained significantly elevated (OR=6.7) when controlled for wood dust exposure. Of interest to this review, Hardell et al. observed a significantly increased odds ratio (OR=5.80, $p<0.05$) between nasal cancer and work in particleboard production. It is not known whether this observation is confounded by wood dust exposure, which can occur in particleboard production.

24. Olsen et al. (1984) of the Danish Cancer Registry conducted a case-control study of nasal cancers. This study examined 839 cancer registry cases (560 males, 279 females), diagnosed between the years 1970-1982, who were matched with 2,467 controls with cancer of the colon, rectum, prostate, and breast on age-sex-year of diagnosis. The researchers used a nationwide data linkage system which has linked cancer cases and previous employment. Occupational histories came from the National Supplementary Pension fund, established in 1964, and the Central Population Registry. Use of these national data sets eliminated the potential for recall bias since cases and controls were not interviewed. In this case-control study, Olsen et al. tested for associations between HCHO, wood dust, paint-lacquer-glue, and metal exposure and sino-nasal cancers. Significantly increased risks were found for nasal cavity cancer for exposure to HCHO (OR=2.8, 95%

CI:1.8-4.3), wood dust (OR=2.5, 95% CI:1.8-3.9), and paint-lacquer-glue (OR=2.1, 95% CI:1.4-3.0). Exposure to both wood dust and HCHO can occur simultaneously, and Olsen et al. performed a stratified analysis which controlled for wood dust exposure. In this analysis, the elevated risk with HCHO exposure was reduced to 1.6 and became nonsignificant. In this stratified analysis, both HCHO and wood dust exposure together resulted in an additive risk (OR=4.1, $p<0.05$, 95% CI:2.3-7.3).

25. Hayes et al. (1986), formerly of the Erasmus University of Rotterdam, presented findings of a case-control study of nose and nasal sinus tumors at the 3rd International Conference on Epidemiology and Occupational Health in Dublin, Ireland. In their published study, Hayes et al. (1986) identified factors associated with 144 cases of nasal and nasal neoplasms diagnosed between 1978 and 1981. Living and deceased population controls were used as the comparison group. Hayes et al. observed significant associations between male adenocarcinoma cases and work in furniture and cabinet making (OR=120, 90% CI:30.9-613.2) and joinery (OR=16, 90% CI:2.8-85.3). Nonsignificantly elevated risks were reported for all cell-types and employment in leather (OR=2.3*), metal (OR=2.1*), and floriculture (OR=3.7, 90% CI:0.9-18.1) industries. In addition, Hayes et al. noted significantly increased risks between nonadenocarcinomas

*90% confidence intervals not reported.

and paint (OR=4.1, 90% CI:1.5-11.2), benzene (OR=2.3, 90% CI:1.4-3.8), and HCHO (OR=2.2, 90% CI:1.2-4.0) exposure.

In analyses examining HCHO exposures among male study subjects with no or low levels of wood dust exposure, different results were observed using 2 independent assessments, A and B, for HCHO exposure. By classification A, a significant excess risk (OR=2.5*, 90% CI:1.2 - 5.0, 15 cases) was observed with HCHO exposure. The authors stated there appeared to be a dose response relationship with level of exposure. By classification B, the odds ratio was reduced to 1.6 and was not statistically significant (90% CI:0.9 - 2.8, 24 cases). The authors attempted analyses of this type for males subjects with high level of wood dust exposure using classification B data. In this analysis, the authors observed an apparent elevation of the odds ratio (OR=1.9, 90% CI:0.7 - 5.5, 15 cases).

EPA has analyzed the data from Classification A for male subjects with no or low levels of wood dust exposure and with high levels of wood dust exposure. In this analysis, the weighted odds ratio for HCHO exposure was significant elevated (OR=1.9, 95% CI:1.1 - 3.8).

Additionally, the authors derived HCHO risks for those classified as high wood dust exposed on both assessments with respect to the wood dust exposure. A statistically significant elevation (odds ratio) in

nasal cavity cancer risk (all tumors: 3.4, 90% CI: 1.1-10.4; squamous cell carcinoma:3.8, 90% CI: 1.1-13.0) was observed. There was, also, a significant ($p<0.05$) association for trend with time since first exposed for all tumor types and for squamous cell carcinomas.

26. Roush et al. (1985) reported at the 1985 Meetings of the Society for Epidemiologic Research results of a case-control study of nasal sinus and nasopharyngeal cancers. This study is yet to be published. At the time of the SER presentation, 198 sinonasal and 173 nasopharyngeal cancer cases had been identified, for the past 41 years, from the Connecticut Tumor Registry. Controls (n=608) were sampled from death certificates. The authors state that occupational histories were derived from city directories and from death certificates.

Apparent elevations in the odds ratio for combined sinonasal and nasopharyngeal cancer (OR=2.2, 95% CI:0.7-7.0) were reported for work in the rubber industry and printing (OR=1.2, 95% CI:0.4-3.7), morticians (OR=2.1, 95% CI:0.7-6.5), and for physicians and dentists (OR=1.5, 95% CI:0.5-5.1). All 4 categories were identified as having potential formaldehyde exposure. In analyses that examined occupational formaldehyde exposure, 20 or more years prior to death, no associations were observed for those cases and controls over 68 years of age between either sinonasal cancer or

nasopharyngeal cancer. For those cases and controls less than 68 years of age, apparent elevations in the odds ratio were observed for both sinonasal cancer (OR=1.4, 95% CI:0.6-3.0) and nasopharyngeal cancer (OR=1.7, 95% CI:0.9-3.5).

Use of city directories and death certificates to ascertain occupational histories lacks sensitivity and may potentially introduce bias.

27. Partenan et al. (1985) conducted a nested case-control study of 60 respiratory cancer cases among male production workers in the wood industry and in formaldehyde glue manufacturing. Respiratory cancers were defined as mouth (other), tongue, pharynx, nasal sinuses, larynx, and lung (trachea). Three referents alive at the time of diagnosis of the corresponding case were selected as controls. These referents were matched to each case by year of birth. Smoking histories were obtained for both cases and controls by mailed questionnaires or by interview.

Smoking and survival status were controlled for in the analyses since more complete work histories were obtained for subjects who were alive at the time of data collection. In addition, HCHO exposure was assessed through the use of a job exposure matrix. Odds ratios were calculated for 1) cumulative HCHO exposure $\geq$3 ppm-months 2) cumulative HCHO exposure $\geq$3 ppm-months, $\geq$10 year latency, 3) peak exposure >2 ppm, 4) peak exposure.

>2 ppm, ≥10 year latency, 5) HCHO-wood dust exposure ≥1 month, 6) HCHO-wood dust exposure ≥1 month, ≥10 years latency, and 7) "ever exposed."

Results of the analyses showed apparent elevations in the odds ratio with cumulative HCHO exposure which accounted for a 10 year latency (OR = 1.6, 8 cases) and with "ever exposed" (OR = 1.52, 55 cases). The exposure-response relation between HCHO exposure and respiratory cancer was analyzed through the classification of levels-of-exposure. In these analyses, only the duration of exposure to HCHO-containing wood dust suggested a positive exposure-response relationship (1 month - 5 years; OR = 0.78, 4 cases; >5 years, OR = 1.82, 6 cases).

This study is limited by low power and a short follow-up period; having an additional effect of lowering the power. Thus, only very large excesses in human risks can be ruled out.

28. Vaughan et al. (1986a,b; and as reported in SAIC, 1986) of the Fred Hutchinson Cancer Research Center, University of Washington, conducted a population-based case-control study of sinonasal and pharyngeal cancers and possible associations with HCHO. This study was composed of 53 sinonasal cases, 27 nasopharyngeal cases, and 205 oro-hypo-pharyngeal cases which were identified from a tumor registry. Controls (n=557) were selected from the general population and were matched to cases on

sex and age. The cases were identified between 1979 and 1983, and interviews were conducted in 1983. Due to a short survival time between diagnosis and death from these neoplasms, next-of-kin (NOK) interviews were obtained for 50% of the cases. Comparison of cases and controls showed that the control population appeared to have a higher educational level than the cases, but this difference was not statistically significant.

Vaughan et al. examined personal, occupational, and environmental HCHO exposures using logistic regression analyses. Formaldehyde exposure was directly assessed by three measures: 1) maximum exposure level, 2) number of years in a formaldehyde job, and 3) weighted sum of years in a formaldehyde job. Formaldehyde exposure was indirectly assessed by identifying occupational and domestic environments (mobile home residency, remodeling, occupational resin-glue-adhesive exposure, etc.) where formaldehyde had been previously reported.

Analyses showed that smoking and alcohol were significantly associated with sinonasal cancer. When occupational and environmental exposures were examined, after control for smoking and alcohol consumption, the odds ratio for exposure (>10,000 hours) to resins, glues, and adhesives (OR = 3.8, 4 cases $p \leq 0.05$) was significantly elevated and the odds ratio with sawdust exposure appeared elevated (OR = 2.4, 8 cases, $p \leq 0.10$). The trends with increasing exposure were

significant for both exposures. No association was observed with the direct assessment of formaldehyde exposure; the odds ratios were below 1.0.

In analyses of the nasopharyngeal cases, Vaughan et al. observed significant associations with smoking and race. After controlling for these variables, significant elevations in the odds ratio were observed with occupational exposure to stains, varnishes, and solvents (OR = 4.0, $p<0.05$) and with ever having lived in a mobile home (OR = 3.0, 8 cases, $p<0.05$), with the highest odds ratio observed for living 10 or more years in a mobile home (OR = 5.5, 4 cases, $p<0.05$). Trends for both exposures were statistically significant. An association with formaldehyde exposure (as assessed from the job linkage system) appeared present since the odds ratios for formaldehyde exposure were above 1.0 and they increased with increasing exposure, but these conclusions are based on a total of 11 cases and were not statistically significant.

Analyses of the oro-hypopharyngeal cases showed significant associations with cigarette and alcohol use, and an alcohol-sex interaction. After considering these variables, significant elevations of the odds ratio were observed with exposure (>10,000 hours) with resins, glues, and adhesives (OR = 3.9, $p<0.05$), stains, varnishes, and solvents (OR = 3.9, $p<0.05$), and asbestos (OR = 4.0, $p<0.05$). Formaldehyde exposure (as assessed

from the job linkage system) was not significantly associated with neoplasms of these sites. Analyses which relied on self-reporting by eliminating next-of-kin interviews showed an elevated odds ratio between formaldehyde exposure, greater than 20 years, and oropharyngeal cancer (OR = 2.0, 95% C.I.:0.9-4.6).

Mobile home residency and occupational resin, glue, and adhesive exposure were among the exposure variables a priori selected as surrogates for formaldehyde exposure. Urea-formaldehyde resins have been used in hardwood plywood for over 35 years (HPMA, 1984) and in particleboard. Hardwood plywood used as prefinished wall panels saw tremendous growth in the 1950's and 1960's, coinciding with the growth of the mobile home industry (HPMA, 1984).

Several of the nasopharyngeal cancer cases who were identified in this study as living in a mobile home lived in what is generally called a recreational vehicle. The interpretation of Vaughan's results changes little because manufacturing practices for mobile homes and for recreational vehicles were very similar; formaldehyde-containing products were used in both. By its retrospective nature, case-control study is limited in its ability to identify if the cases had a specific exposure and, if so, at what level of exposure.

A second limitation of this study is the accuracy of the NOK interviews. Fifty percent of the cases were

dead at the time of the interview and next-of-kin may not remember all occupational histories, although one expects that residential history may be more clearly remembered. Inclusion of NOK interviews potentially biases the results towards the null hypothesis of no effect. There is support for the presence of such a bias from analyses which eliminated NOK interviews. The resultant odds ratios in these analyses were larger than those odds ratios in analyses which included both live and NOK interviews. It must be noted that the results from NOK-eliminated analyses were not statistically significant; a possible reflection of reduced number of cases and, hence, reduced power. Findings which are not statistically significant, therefore, may be due to the third limitation of this study, reduced power to detect very small elevations in risk.

APPENDIX 3: ESTIMATES OF RISK USING VARIOUS EXTRAPOLATION MODELS

Appendix 3

Eleven models were used to extrapolate risks from the CIIT rat malignant tumor data. All were dichotomous ("tumor/no tumor" or quantal) models. The formulation of each model to accommodate quantal response data was preferred to one including time as a variable. Simulation studies conducted for the EPA indicated that inclusion of time as a variable would not provide much improvement in estimation (Howe et al., 1984). Additionally, the lack of information about causes of death of experimental animals and the adjustments made for sacrifice data would have necessitated assumptions that could have brought the validity of results based on time into question.

Table 1 shows the parameter estimates (with standard errors), log-likelihood, and X^2 goodness-of-fit test statistics (with p-values) for the eleven models: one-, three-, and five- stage models and additive and independent background forms of the probit, logit, Weibull, and gamma-multihit models.

Tables 2 through 6 give the maximum likelihood estimates (MLE) and upper bound estimates for selected occupational exposures. The shapes of most models' upper-bound estimates tend to parallel the shapes of the models themselves, unless a procedure has been devised to provide otherwise. This is the case for the linearized multistage procedure, which provides a linear upper bound estimate for a multistage model at low doses. The MLE, which is the estimate given by a fitted model, takes only the experiment to which the model has been fitted into account. The upper bound estimate is intended to account for experiment to experiment variability as well as extrapolation uncertainties.

Table 1. Extrapolation Model Statistics

Model	Parameters (Standard Error)			Loglikelihood[a]	χ^2 Goodness-of-Fit (Significance Level)[b]
Independent Background	α	β	γ		
Probit	-7.13	2.85	0.00	-99.30	<0.001
	(0.80)	(0.31)	(0.00)		(0.996)
Logit	-13.61	5.38	0.00	-99.31	0.008
	(2.04)	(0.78)	(0.00)		(0.927)
Weibull	-12.53	4.75	0.00	-99.32	0.016
	(2.00)	(0.76)	(0.00)		(0.899)
Gamma	0.79	10.19	0.00	-99.31	0.001
	(0.17)	(2.21)	(0.00)		(0.979)
Additive Background	α	β	δ		
Probit	-14.62	4.96	6.59	-99.31	0.006
	(61.49)	(16.23)	(51.33)		(0.937)
Logit	-15.46	5.95	0.87	-99.32	0.019
	(25.51)	(7.66)	(12.04)		(0.892)
Weibull	-22.67	7.66	5.31	-99.41	0.109
	(42.31)	(11.51)	(20.74)		(0.741)
Gamma	0.79	10.19	0.00	-99.30	0.001
	(2.52)	(52.06)	(24.98)		(0.979)

Multistage	q_0	q_1	q_2	q_3	q_4	q_5		
5-Stage	0.0	0.0	0.0	0.0	4.34E-6	1.56E-6	-99.32	0.020 (0.99)[c]
3-Stage	0.0	0.0	0.0	3.46E-4	-	-	-104.30	6.98 (<.1)[c]
1-Stage	0.0	3.94E-2	-	-	-	-	-154.17	79.05 (<.0001)[c]

[a] The closer the loglikelihood to zero the better the model fits the observed data.

[b] Significance levels less than 0.01 indicate an inadequate fit.

[c] Approximately distributed as a chi square with degrees of freedom equal to the number of doses minus the number of nonzero parameter estimates.

Table 2. Risks to Mobile Home Residents (HUD Standard)

Level of Exposure: 0.15 ppm, 112 hours/week, 10 years

Model	Point Estimate of Added Risk	95% Upper Confidence Limit on Added Risk
Independent Background		
Probit	0.0	0.0
Logit	2×10^{-11}	2×10^{-10}
Weibull	2×10^{-10}	2×10^{-9}
Gamma	3×10^{-17}	2×10^{-16}
Additive Background		
Probit	3×10^{-8}	2×10^{-6}
Logit	7×10^{-8}	4×10^{-6}
Weibull	6×10^{-6}	5×10^{-5}
Gamma	3×10^{-17}	8×10^{-14}
5-Stage Multistage	1×10^{-9}	2×10^{-4}
3-Stage Multistage	6×10^{-4}	1×10^{-4}
1-Stage Multistage	3×10^{-3}	4×10^{-3}

Table 3. Risks to Manufacturers of Apparel (OSHA Standard)

Level of Exposure: 3.0 ppm, 36 hours/week, 40 years

Model	Point Estimate of Added Risk	95% Upper Confidence Limit on Added Risk
Independent Background		
Probit	2×10^{-5}	9×10^{-5}
Logit	3×10^{-4}	9×10^{-4}
Weibull	5×10^{-4}	1×10^{-3}
Gamma	1×10^{-4}	4×10^{-4}
Additive Background		
Probit	2×10^{-4}	3×10^{-3}
Logit	4×10^{-4}	3×10^{-3}
Weibull	1×10^{-3}	3×10^{-3}
Gamma	1×10^{-4}	2×10^{-3}
5-Stage Multistage	5×10^{-4}	6×10^{-3}
3-Stage Multistage	6×10^{-3}	9×10^{-3}
1-Stage Multistage	8×10^{-2}	9×10^{-2}

Table 4. Risks to Manufacturers of Apparel (Personal Sample)

Level of Exposure: 0.64 ppm, 36 hours/week, 40 years

Model	Point Estimate of Added Risk	95% Upper Confidence Limit on Added Risk
Independent Background		
Probit	8×10^{-17}	3×10^{-16}
Logit	8×10^{-8}	4×10^{-7}
Weibull	3×10^{-7}	1×10^{-6}
Gamma	7×10^{-11}	6×10^{-10}
Additive Background		
Probit	5×10^{-7}	2×10^{-5}
Logit	1×10^{-6}	6×10^{-5}
Weibull	5×10^{-5}	3×10^{-4}
Gamma	7×10^{-11}	3×10^{-8}
5-Stage Multistage	6×10^{-7}	1×10^{-3}
3-Stage Multistage	6×10^{-5}	7×10^{-4}
1-Stage Multistage	2×10^{-2}	2×10^{-2}

Table 5. Risks to Manufacturers of Apparel (Area Sample)

Level of Exposure: 0.23 ppm, 36 hours/week, 40 years

Model	Point Estimate of Added Risk	95% Upper Confidence Limit on Added Risk
Independent Background		
Probit	0.0	1×10^{-28}
Logit	3×10^{-18}	2×10^{-9}
Weibull	2×10^{-9}	1×10^{-8}
Gamma	3×10^{-15}	4×10^{-14}
Additive Background		
Probit	7×10^{-8}	4×10^{-6}
Logit	2×10^{-7}	1×10^{-5}
Weibull	1×10^{-5}	1×10^{-4}
Gamma	3×10^{-15}	4×10^{-12}
5-Stage Multistage	9×10^{-9}	4×10^{-4}
3-Stage Multistage	3×10^{-6}	2×10^{-4}
1-Stage Multistage	6×10^{-3}	7×10^{-3}

Table 6. Risks to Manufacturers of Apparel (NIOSH Data)

Level of Exposure: 0.17 ppm, 36 hours/week, 40 years

Model	Point Estimate of Added Risk	95% Upper Confidence Limit on Added Risk
Independent Background		
Probit	0.0	0.0
Logit	6×10^{-11}	4×10^{-10}
Weibull	5×10^{-10}	4×10^{-9}
Gamma	1×10^{-16}	2×10^{-15}
Additive Background		
Probit	4×10^{-8}	3×10^{-6}
Logit	1×10^{-7}	7×10^{-6}
Weibull	9×10^{-6}	8×10^{-5}
Gamma	1×10^{-16}	3×10^{-13}
5-Stage Multistage	3×10^{-9}	3×10^{-4}
3-Stage Multistage	1×10^{-6}	2×10^{-4}
1-Stage Multistage	5×10^{-3}	5×10^{-3}

APPENDIX 4: DOCUMENTATION OF HIGH TO LOW DOSE EXTRAPOLATION MODELS USED IN QUANTITATIVE RISK ASSESSMENT-CONCISE DESCRIPTION

TABLE OF CONTENTS

1.0 INTRODUCTION

The Design and Development Branch (DDB) of the Exposure Evaluation Division (EED) of the U.S. Environmental Protection Agency currently uses six extrapolation models to estimate carcinogenic risk in humans from animal test data. This report provides general introductory material and a concise description of each model suitable for insertion into the background and methods section of a quantitative risk assessment. No mathematical equations are included and technical terms are avoided as much as possible. The introductory material covers the reason for high- to low-dose extrapolation, the type of data used, classes of models and incorporation of spontaneous background response. The six models that are described are the probit, logit, Weibull, one-hit, gamma-multihit and multistage models. Technical details and a more comprehensive bibliography appear in a companion report (Chesson and Zanetos, 1983).

2.0 GENERAL INFORMATION

2.1 THE NEED FOR HIGH- TO LOW-DOSE EXTRAPOLATION

The most readily available source of information for determining the health effects of toxic agents comes from experimental tests on laboratory animals. In order to obtain observable effects within a reasonable time the laboratory animals must be exposed to concentrations, or doses, of the toxic substance that are higher than those expected to be experienced by humans. Therefore it is necessary to predict the effects at low doses from the effects observed at higher doses. This process is called "high- to low-dose extrapolation" and is carried out by fitting a mathematical model to the observed data and using the model to estimate the effect of the substance at low doses.

2.2 TYPE OF DATA

A typical experiment to collect data for high- to low-dose extrapolation consists of several groups of animals. Each group is exposed to a different dose, or concentration d, of the agent under test and the numbers of animals in each group that show a particular response within a fixed time period are recorded. This type of data is often called "quantal" response or "dichotomous" data. The data provide the basis for fitting dose response models and extrapolating to low doses. As used here, a dose response model is a mathematical relationship between the applied dose d, and the proportion of animals in the group, P(d), that show the response. When additional information, such as the time between the start of exposure to the agent and appearance of

a tumour, is available then other types of dose response models may also be used.

2.3 TYPES OF MODELS FOR QUANTAL RESPONSE DATA

The mathematical models used for describing quantal responses fall into two broad types:

- Tolerance distribution models
- Stochastic or mechanistic models.

(Krewski and Van Ryzin, 1981; Brown, 1983).

Tolerance distribution models are based on the idea that each individual in a population has its own tolerance to the test agent. If a dose does not exceed the tolerance of an individual then there will be no response by that individual. If the dose exceeds the tolerance then a response will be observed. Tolerance models differ from each other in the particular mathematical expression used to describe the distribution of tolerances in the population (Chand and Hoel, 1974). The distributions are generally chosen because of their descriptive power rather than on the basis of biological processes.

Stochastic, or mechanistic, models are derived from plausible biological arguments which lead to an expression for $P(d)$, the expected proportion of animals that will show a response at dose d. Sometimes the mechanistic argument leads to a tolerance distribution. Thus the distinction between the two types of model is not always very obvious.

The behavior of a model at low doses is of particular importance since it is the low dose region about which predictions are to be made. In this region the shape of the dose response curve predicted by a model may be convex, linear or concave (Figure 1). The usual procedure is to estimate the dose d*, corresponding to a particular low level of risk having taken into account spontaneous background response (see Section 2.4 below). The excess risk is commonly set at 10^{-6}, i.e. the level at which the expected proportion of individuals that will show the response as a result of exposure to dose d* is one in 1,000,000. The dose d* is called the "virtually safe dose" (VSD). Figure 1 shows how the estimate of the VSD depends on the shape of the dose response curve. A linear curve will give a lower VSD than a comparable convex curve and a higher VSD than a concave curve. These relationships are used in some extrapolation procedures that attempt to place a lower bound on the VSD rather than obtain an actual estimate of it. The estimates obtained in this way are considered 'conservative' in that they are expected to overestimate risk and underestimate the VSD.

2.4 SPONTANEOUS BACKGROUND RESPONSE

In an experiment to determine a dose response curve it is possible that some animals might show a response even though they receive zero dose of the chemical. This spontaneous background response may be a result of many factors including the

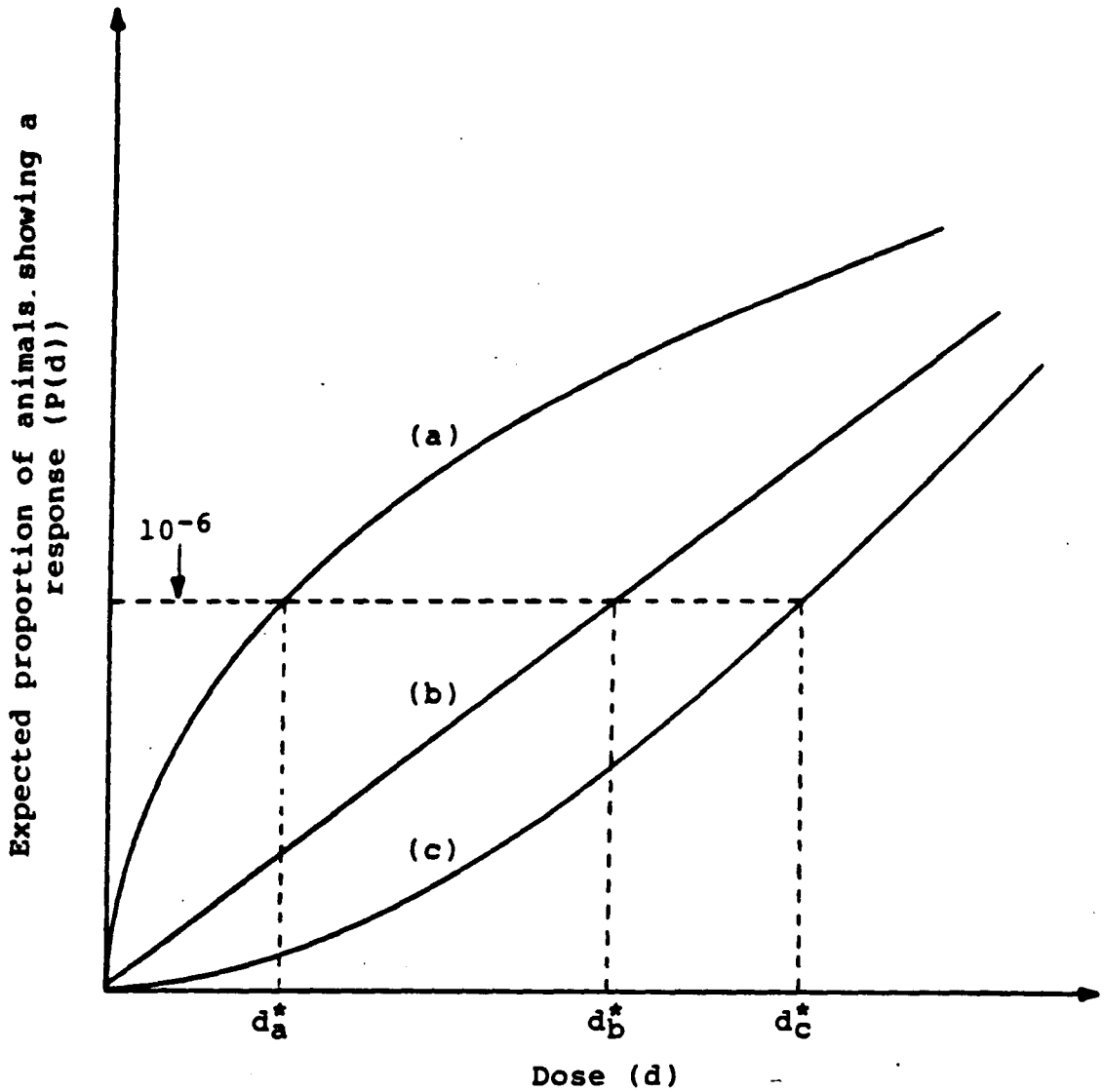

FIGURE 1. THE RELATIONSHIP BETWEEN THE SHAPE OF THE DOSE RESPONSE CURVE AT LOW DOSES AND THE VSD (d*) (ASSUMING NO SPONTANEOUS BACKGROUND RESPONSE)

(a) Concave
(b) Linear
(c) Convex

presence of another response causing chemical, the genetic make-up of the strain of animal, or a background level of the toxic substance which is present in the environment. The method of incorporating the spontaneous background response into the model affects the shape of the dose response curve at low doses and hence estimates of the VSD.

If the background response is assumed to be totally independent of the response to the experimental dose then the shape of the dose response curve remains qualitatively the same as when no background response is included. However, if the background response is assumed to be additive in the sense that the effective dose is taken to be the background dose plus the experimental dose then in many cases the dose response curve becomes linear at low doses (Crump et al, 1976). This is true for most of the commonly used models, including the probit, logit, Weibull, one-hit, gamma-multihit and multistage models. A dose response curve that is linear at low doses will result in a smaller VSD than a comparable dose response curve that is convex at low doses. Thus, a model that includes additive background effects is likely to give estimates of the VSD which are smaller than those estimated by a model without additive background.

A model may include both independent and additive background but as long as the additive component is non-zero the dose response curve at low doses will be linear (Crump et al, 1976; Peto, 1978).

It is often difficult to decide from experimental data whether background responses are independent, additive, or both

(Brown, 1983). Therefore, it has been suggested that models incorporating additive background be used unless there is good evidence to assume otherwise (State of California, Health and Welfare Agency, 1982).

3.0 PROBIT MODEL (LOGNORMAL)

The probit model is a tolerance distribution model in which the distribution of individual tolerances is assumed to follow a lognormal distribution. The dose response curve is S-shaped and symmetric about the 50 percent level. Since experimental data often show this sort of pattern, the probit model has been used extensively in toxicological studies and a standard statistical theory has been developed around it (Finney, 1971). The use of the probit model to extrapolate to low doses is more recent.

When the background response is independent of the induced response the probit model approaches zero very rapidly as dose decreases, more rapidly than other common models used in low-dose extrapolation (Krewski and Van Ryzin, 1981). This means that the probit model with independent background cannot be linear at low doses and tends to give lower estimates of risk and higher VSD's than those obtained from other models, or from linear extrapolation.

The Mantel-Bryan procedure (Mantel and Bryan, 1961; Mantel et al, 1975) uses the probit model to obtain a "conservative" estimate of the VSD. By taking an arbitrary value of 1 for the slope parameter of the model and extrapolating from the range of observable responses it is assumed that the extrapolated curve will lie above the true curve and therefore provide a conservative estimate of a VSD. However the validity of this procedure has been questioned (Crump, 1977). In particular, it is not

clear that taking the value of the slope parameter as 1 is necessarily conservative (Connfield et al, 1978).

4.0 LOGIT MODEL (LOG LOGISTIC MODEL)

The logit model is a tolerance distribution model which has a very similar appearance to the probit model within the range of observable responses. It is S-shaped and symmetric about the 50 percent response level. However it differs from the probit model in that at low doses it approaches zero much more slowly. The model can be derived from chemical kinetic theory and was proposed as a dose response model by Worcester and Wilson (1943) and Berkson (1944).

With independent background the excess risk may be linear, convex or concave at low doses. Low-dose linearity implies a concave dose response curve at higher doses. With additive background the excess risk will always be linear at low doses (Peto, 1978). Therefore, linear extrapolation procedures will tend to give estimates of VSD that are close to or smaller than those based on the model itself unless the dose response curve is concave at low doses. In fitting a variety of models to 20 data sets Krewski and Van Ryzin (1981) found that estimates of VSD based on the logit model were smaller than those based on the probit model and similar to those based on the gamma-multihit model.

5.0 WEIBULL MODEL (EXTREME VALUE)

The Weibull model is a tolerance distribution model which is suggested by human cancer incidence patterns (Cooke et al, 1979). Pike (1966) showed that two quite general assumptions, (1) cancer begins in a single cell, and (2) individual cells behave independently, can lead to a Weibull distribution for tolerances. The model can also be derived from a "time-to-tumour" argument (Peto et al, 1972) or from a model based on critical cell clusters (Scott and Hahn, 1980). Generalized forms of the Weibull model are discussed by Carlborg (1981a).

With independent background the excess risk at low doses predicted by the Weibull model behaves in the same way as the logit model. The excess risk may be linear, convex or concave depending on the value of the shape parameter. Low dose linearity implies a concave dose response curve at moderate and high doses. With additive background, the excess risk will always be linear at low doses (Peto, 1978).

Estimates of the VSD based on the Weibull model tend to be less conservative than the multistage model but more conservative than the probit, logit and gamma-multihit models (Krewski and Van Ryzin, 1981).

6.0 ONE-HIT MODEL (LINEAR MODEL)

The one-hit model is derived from a mechanistic description of the carcinogenic process. Suppose that there is a response after a susceptible site has been "hit" by a single biologically effective unit of dose within a fixed period of time. By assuming that the number of hits over that time period follows a Poisson distribution and the average number of hits is proportional to the dose, a formula is easily obtained for the probability (P(d)) of obtaining a response at a given dose (d). The Poisson distribution assumption is appropriate when hits occur randomly through time and the occurrence of a hit has no effect on the occurrence of other hits.

In the absence of spontaneous background responses the one-hit model has only one unknown parameter and is always linear at low doses and concave at moderate and high doses. Because of its low dose linearity, it is often referred to as the linear model. It is a special case of the gamma-multihit, multistage and Weibull models.

The one-hit model does not provide a good fit to many sets of empirical data because the model is concave at higher dose levels whereas many data sets are convex. It appears to be appropriate for only one (hexachlorobenzene) of the 20 data sets considered by Krewski and Van Ryzin (1981). However, it has been used extensively in low dose extrapolation as a conservative estimate of risk, assuming that the true dose response curve is likely to be convex at low doses and hence lie below that of the one-hit model (Hoel et al, 1975; BEIR Report, 1972). In some

situations the one-hit model is only fitted to the lowest dose groups where the experimental data are consistent with the model (Altshuler, 1976). The model has been criticized as being unduly conservative in some circumstances. For example, Van Ryzin and Rai (1980) found that for ethylene thiourea the VSD (for risk level 10^{-6}) estimated by the one-hit model was approximately 1/60,600th that of the multihit model and 1/8050th that of the multistage model.

7.0 GAMMA-MULTIHIT MODEL

The gamma-multihit model can be derived from a mechanistic description of the carcinogenic process. Suppose the response to a particular chemical is the result of k biological precursor events or "hits" at a susceptible site within a fixed period of time. By assuming that the number of hits over that time period follows a Poisson distribution and the average number of hits is proprotional to the dose, a formula can be obtained for the probability (P(d)) of obtaining a response at a given dose (d). The Poisson distribution assumption is appropriate when hits occur randomly through time and the occurrence of a hit has no effect on the occurrence of other hits. When only one hit (k=1) is required to cause a response, the model is referred to as the one-hit model.

The gamma-multihit model can also be regarded as a tolerance distribution model since the formula for P(d) is identical to the dose response curve generated by assuming that each individual in the population has a particular tolerance level to the chemical and that the distribution of tolerance levels follows a gamma distribution. In this situation the gamma distribution is merely used to describe the shape of the dose response curve and has no mechanistic implications.

For small doses the dose response curve is concave when $k < 1$, linear if $k=1$ and convex if $k > 1$ (Figure 1). Thus the gamma-multihit model provides a greater variety of behavior at low doses than models which can have only linear or convex dose response curves at low doses. However, $k < 1$ is not easily

interpretable in terms of the mechanistic "hit" model, and one has to resort to the descriptive tolerance distribution interpretation in this case.

Although the gamma-multihit model has been recommended for use in risk assessment calculations (Food Safety Council, 1978), this recommendation has been criticized by Haseman et al (1981) because the model can produce estimates of the VSD which are unrealistically high or unrealistically low in certain situations. These problems appear to be less likely to occur when an additive background effect is included in the model thereby causing the dose response curve to be linear at low doses.

The gamma-multihit model tends to produce estimates of the VSD which are less conservative than the multistage and Weibull models and more conservative than the logit and probit models (Krewski and Van Ryzin, 1981).

8.0 MULTISTAGE MODEL

The multistage model was derived to account for the observation that for many types of cancer, death rate is proportional to some power of age (e.g. Nordling, 1953). The model was developed by Armitage and Doll (1961) and extended by Crump et al (1976). The Armitage and Doll model assumes that a cell line goes through k distinct stages in a specific order before becoming cancerous and that the rate at which it progresses through the ith stage is a constant λ_i. Different cell lines develop independently and the time to cancer is determined by the most rapidly developing line. This model predicts that cancer incidence will increase as $(age)^{k-1}$.

Crump et al (1976) extended the Armitage and Doll model by assuming that λ_i, the rate at which a cell goes through the ith stage, is linearly related to dose. From these assumptions, the probability of a response, P(d), at dose d can be expressed in terms of a polynomial in d. The model is essentially unchanged irrespective of whether indepenedent or additive background responses are assumed. It is linear at low doses if the polynomial in d has a linear term and convex otherwise. It cannot be concave at low doses, but it can be simultaneously linear at low doses and convex at moderate doses. When k=1, i.e., when there is only one stage, the model reduces to the one-hit model.

The multistage model cannot describe concave dose response curves such as those observed with DDT, vinyl chloride, diethylstilbestrol and ethylene dibromide (Carlborg, 1981b).

Also, the shape of the curve in the extrapolated low dose region is relatively insensitive to the shape of the dose response function in the observable range (Carlborg, 1981a).

The parameters of the multistage model are more complicated to estimate than for other models. However, computer programs are available to carry out the calculations (Crump and Watson, 1979; Howe and Crump, 1982).

Crump (1982) proposed a method of linear extrapolation based on the multistage model, which has been adopted by the EPA in setting water quality criteria (USEPA, 1980) and in other areas of risk assessment. The method is an improvement of the extrapolation procedure proposed by Crump et al (1977). It involves approximating the dose response curve by a straight line with slope given by the estimated linear term of the multistage model. This modification has been referred to as the "linearized" multistage model and has an advantage over some other linear extrapolation procedures in that information from all the experimental dose groups is used, not just the lower groups. The GLOBAL79 computer program (Crump and Watson, 1979) calculates confidence limits for the linearized multistage model. A newer program (GLOBAL82, Howe and Crump, 1982) uses a different method to allow valid confidence limits for estimates at higher doses as well as at low doses. At low doses the results obtained from GLOBAL79 and GLOBAL82 should be very similar.

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APPENDIX 5: SENSITIVITY ANALYSIS OF CIIT RAT DATA USING THE LINEARIZED MULTISTAGE MODEL

Appendix 5

Ten perturbations of the final CIIT formaldehyde combined male and female Fischer 344 rat data were constructed in 3 ways: one, as if a dose had not been run; two, as if the response had been different at an intermediate dose; or, three, the response was different at the highest dose or control (see Table 1). Perturbations 1 and 2 removed one intermediate dose and also increased the number of animals at risk by 1, thereby lowering the response rate at the highest dose. Perturbation 3 eliminated the response and number of animals at risk at the high dose entirely. Perturbation 4 gave the CIIT data set a positive response at control. Pertubations 5 and 7 use the correct final denominator or number of animals at risk at the highest dose, but vary the numbers responding. Perturbations 6 and 8 lower or raise the response at the next to the highest dose. Perturbation 9 eliminates the control data entirely. Perturbation 10 gives a positive response at the lowest positive dose where one did not appear in the CIIT data set.

For all perturbations, with a five-stage model the fit was adequate (see Table 2, first column). While the individual maximum likelihood coefficients varied from perturbation to perturbation, the predicted upper bound risks varied by less than a factor of 2 (see Table 3).

A graphical representation of the 10 perturbations is given in Figures 1 an 2 which, upon inspection, reveal just how similar the "curves" are. Note in Figure 2 that the curve for perturbation 2 has a considerably steeper slope and is rather an outline. This is because the lowest positive dose point was dropped, a section of the curve where the most information is needed.

Table 1. Sensitivity of the 5-Stage Model
(Model Statistics)

	Dose (ppm) Responding/Tested				Parameter Estimates					
Perturbation	0.0	2.0	5.6	14.3	q_0	q_1	q_2	q_3	q_4	q_5
1	0/156	0/159	-	94/141	0.0	0.0	0.0	0.0	0.0	1.84E-6
2	0/156	-	2/150	94/141	0.0	1.5E-6	5.99E-7	8.00E-7	5.16E-6	1.47E-6
3	0/156	0/159	2/150	-	0.0	0.0	0.0	0.0	0.0	2.42E-6
4	1/156	0/159	2/153	94/140	3.16E-2	0.0	0.0	0.0	0.0	1.86E-6
5	0/156	0/159	2/153	95/140	0.0	0.0	0.0	0.0	4.00E-6	1.62E-6
6	0/156	0/159	1/153	94/140	0.0	0.0	0.0	0.0	0.0	1.85E-6
7	0/156	0/159	2/153	93/140	0.0	0.0	0.0	0.0	4.67E-6	1.50E-6
8	0/156	0/159	3/153	94/140	0.0	0.0	4.06E-10	0.0	1.52E-5	8.01E-7
9	-	0/159	2/153	94/140	0.0	0.0	0.0	0.0	4.34E-6	1.56E-6
10	0/156	1/159	2/153	94/140	0.0	1.68E-3	0.0	0.0	0.0	1.81E-6

Table 2. Sensitivity of the 5-Stage Model
(Model Goodness-of-Fit)

Perturbation	χ^2 Goodness-of-Fit (Significance Level)	Loglikelihood
1	9.35E-8 (7.995)[a]	-89.758
2	1.69E-26 (1.000)	-100.37
3	1.24E-2 (7.995)	-10.634
4	1.04 (7.75)	-106.065
5	1.96E-2 (<.50)	-98.592
6	0.21 (0.98)	-94.794
7	2.07E-2 (0.99)	-100.023
8	4.44E-2 (7.90)	-103.452
9	2.01E-2 (7.90)	-99.34
10	0.69 (7.98)	-105.697

[a] Approximately distributed as a chi square with degrees of freedom equal to the number of doses minus the number of nonzero parameter estimates.

Table 3. Coefficients (q*'s) corresponding to the upper bound on risk for the 5-Stage Model for ten perturbations of the model.

Perturbation Number	q^*_0	q^*_1	q^*_2	q^*_3	q^*_4	q^*_5
1	0.0000	4.2556×10^{-3}	0.0000	0.0000	0.0000	1.7353×10^{-6}
2	0.0000	4.6685×10^{-3}	0.0000	0.0000	0.0000	1.7056×10^{-6}
3	0.0000	3.6499×10^{-3}	0.0000	0.0000	0.0000	0.0000
4	2.5652×10^{-3}	2.3843×10^{-3}	0.0000	0.0000	0.0000	1.7832×10^{-6}
5	0.0000	2.6652×10^{-3}	0.0000	0.0000	0.0000	1.8176×10^{-6}
6	0.0000	1.7512×10^{-3}	0.0000	0.0000	0.0000	1.7966×10^{-6}
7	0.0000	2.7074×10^{-3}	0.0000	0.0000	0.0000	1.7450×10^{-6}
8	0.0000	3.5135×10^{-3}	0.0000	0.0000	0.0000	1.7660×10^{-6}
9	0.0000	2.6866×10^{-3}	0.0000	0.0000	0.0000	1.7809×10^{-6}
10	0.0000	4.9145×10^{-3}	0.0000	0.0000	0.0000	1.7220×10^{-6}

Figure 1. Sensitivity of the 5-Stage Model for all ten perturbations of the dose-response data for Fischer 344 rats.

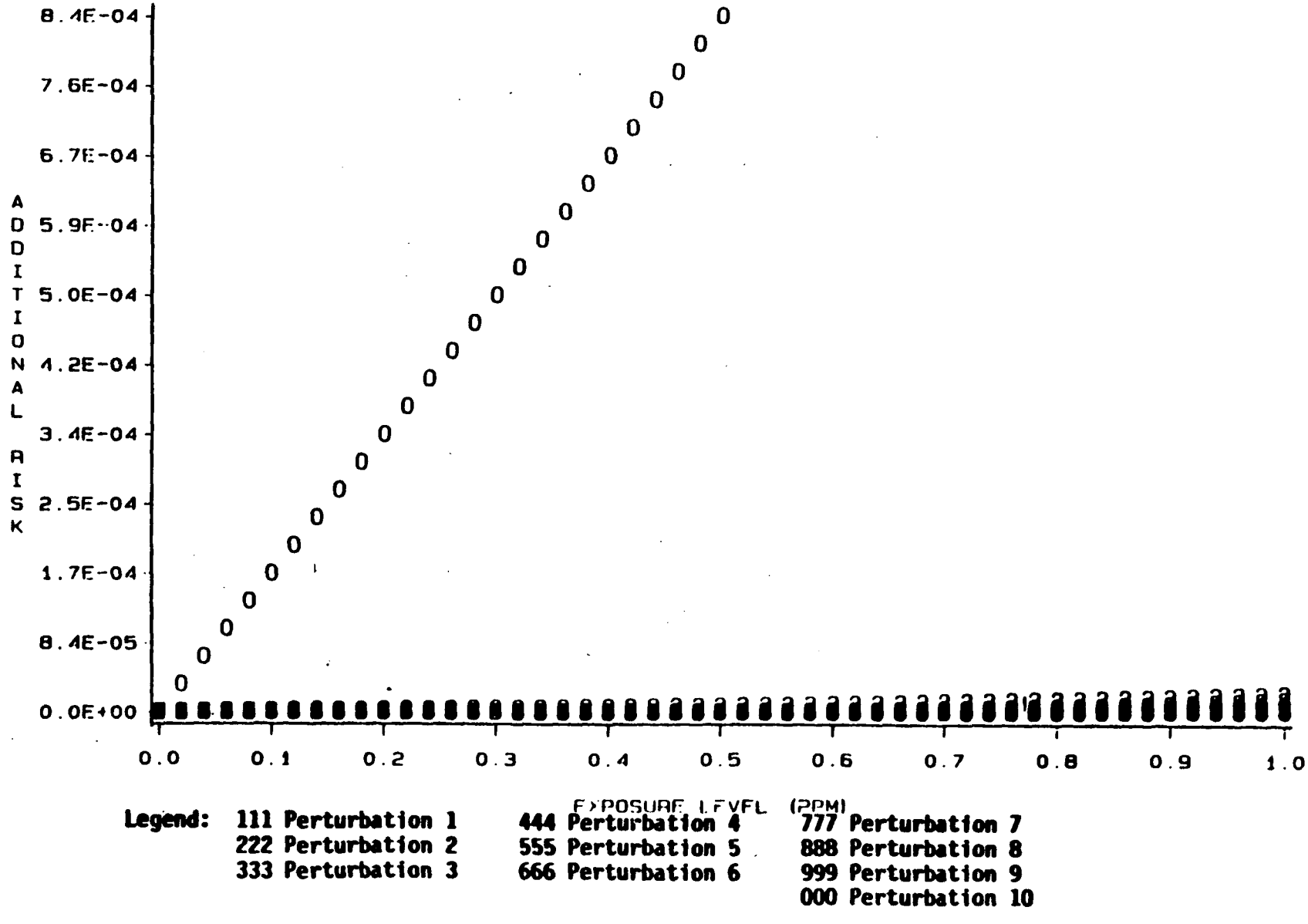

Figure 2. Sensitivity of the 5-Stage Model rescaled to display perturbations 1-9 of the dose-response data for Fischer 344 rats.