TOXICOLOGICAL REVIEW OF

FORMALDEHYDE -

INHALATION ASSESSMENT

(CAS No. 50-00-0)

In Support of Summary Information on the
Integrated Risk Information System (IRIS)

VOLUME I of IV

Introduction, Background,
and Toxicokinetics

June 2, 2010

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<td>American Conference of Governmental Industrial Hygienists</td>
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<tr>
<td>ADAF</td>
<td>age-dependent adjustment factors</td>
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<td>ADH</td>
<td>alcohol dehydrogenase</td>
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<td>ADS</td>
<td>anterior dorsal septum</td>
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<td>AGT</td>
<td>O&lt;sup&gt;6&lt;/sup&gt;-alkylguanyl-DNA alkyltransferase</td>
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<td>AIC</td>
<td>Akaike Information Criterion</td>
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<tr>
<td>AIE</td>
<td>average intensity of exposure</td>
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<td>ALB</td>
<td>albumin</td>
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<td>ANOVA</td>
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<td>aspartate aminotransferase</td>
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<td>airborne toxic control measure</td>
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<td>American Thoracic Society</td>
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<td>Agency for Toxic Substances and Disease Registry</td>
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<tr>
<td>AUC</td>
<td>area under the curve</td>
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<td>confidence interval</td>
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<td>Chemical Industry Institute of Toxicology</td>
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<td>CLL</td>
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<td>CML</td>
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<td>CNS</td>
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<td>CO₂</td>
<td>carbon dioxide</td>
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<td>COEHHA</td>
<td>California Office of Environmental Health Hazard Assessment</td>
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<td>CREB</td>
<td>cyclic AMP responsive element binding proteins</td>
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<tr>
<td>CS</td>
<td>conditioned stimulus</td>
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<tr>
<td>C × t</td>
<td>concentration times time</td>
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<td>Daltons</td>
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<td>daily exposure index</td>
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<td>diethylnitrosamine</td>
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<tr>
<td>Der f</td>
<td>common dust mite allergen</td>
</tr>
<tr>
<td>DMG</td>
<td>dimethylglycine</td>
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<tr>
<td>DMGDH</td>
<td>dimethylglycine dehydrogenase</td>
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<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>EC</td>
<td>effective concentration</td>
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<td>Environmental Health Committee</td>
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<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<td>EPA</td>
<td>U.S. Environmental Protection Agency</td>
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<td>ERPG</td>
<td>emergency response planning guideline</td>
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<td>ethmoid turbinates</td>
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<td>formaldehyde dehydrogenase</td>
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<td>FDA</td>
<td>U.S. Food and Drug Administration</td>
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<td>FDR</td>
<td>fecundability density ratio</td>
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<td>FEF</td>
<td>forced expiratory flow</td>
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<td>FEMA</td>
<td>Federal Emergency Management Agency</td>
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<tr>
<td>FEV₁</td>
<td>forced expiratory volume in 1 second</td>
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<td>FISH</td>
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<td>FSH</td>
<td>follicle-stimulating hormone</td>
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<td>FVC</td>
<td>forced vital capacity</td>
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<td>GALT</td>
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<td>GC-MS</td>
<td>gas chromatography-mass spectrometry</td>
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<tr>
<td>GD</td>
<td>gestation day</td>
</tr>
<tr>
<td>GI</td>
<td>gastrointestinal</td>
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<td>GO</td>
<td>gene ontology</td>
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<td>G6PDH</td>
<td>glucose-6-phosphate dehydrogenase</td>
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<td>GPX</td>
<td>glutathione peroxidase</td>
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<tr>
<td>GR</td>
<td>glutathione reductase</td>
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<tr>
<td>GM-CSF</td>
<td>granulocyte macrophage-colony-stimulating factor</td>
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<td>GSH</td>
<td>reduced glutathione</td>
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<td>GSNO</td>
<td>S-nitrosoglutathione</td>
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<td>GST</td>
<td>glutathione S-transferase</td>
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<td>HAP</td>
<td>hazardous air pollutant</td>
</tr>
<tr>
<td>Hb</td>
<td>hemoglobin</td>
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<td>HCl</td>
<td>hydrochloric acid</td>
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<td>hematocrit</td>
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<tr>
<td>HEC</td>
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<td>5-HIAA</td>
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<td>hm</td>
<td>hydroxymethyl</td>
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<td>HMGSH</td>
<td>S-hydroxymethylglutathione</td>
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<tr>
<td>HPA</td>
<td>hypothalamic-pituitary adrenal</td>
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<td>hypothalamo-pituitary-gonadal</td>
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<td>HPLC</td>
<td>high-performance liquid chromatography</td>
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<td>HPRT</td>
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<tr>
<td>HR</td>
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<td>HSA</td>
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<td>HSDB</td>
<td>Hazardous Substances Data Bank</td>
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<td>Hsp</td>
<td>heat shock protein</td>
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<td>HUVEC</td>
<td>human umbilical vein</td>
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<td>Description</td>
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<tr>
<td>endothelial cell</td>
<td>MEF</td>
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<td>I cell</td>
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<td>IARC</td>
<td>International Agency for Research on Cancer</td>
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<td>ICD</td>
<td>International Classification of Diseases</td>
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<td>IPCS</td>
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<td>IRIS</td>
<td>Integrated Risk Information System</td>
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<td>K_m</td>
<td>Michaels-Menton constant</td>
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<td>LDH</td>
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<td>LEC</td>
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<td>MDA</td>
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<td>4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone</td>
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<td>N\textsuperscript{6}-hmdA</td>
<td>N\textsuperscript{6}-hydroxymethyldeoxyadenosine</td>
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<td>NICNAS</td>
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<td>PMR</td>
<td>proportionate mortality ratio</td>
</tr>
<tr>
<td>PMS</td>
<td>posterior medial septum</td>
</tr>
<tr>
<td>PND</td>
<td>postnatal day</td>
</tr>
<tr>
<td>POD</td>
<td>point of departure</td>
</tr>
<tr>
<td>POE</td>
<td>portal of entry</td>
</tr>
<tr>
<td>PTZ</td>
<td>pentileternetetrazole</td>
</tr>
<tr>
<td>PUFA</td>
<td>polyunsaturated fatty acids</td>
</tr>
<tr>
<td>PWULLI</td>
<td>population weighted unit length labeling index</td>
</tr>
<tr>
<td>QM</td>
<td>quinolinic acid</td>
</tr>
<tr>
<td>RA</td>
<td>reflex apnea</td>
</tr>
<tr>
<td>RANTES</td>
<td>regulated upon activation, normal T-cell expressed and secreted</td>
</tr>
<tr>
<td>RB</td>
<td>reflex bradypnea</td>
</tr>
<tr>
<td>RBC</td>
<td>red blood cells</td>
</tr>
<tr>
<td>REL</td>
<td>recommended exposure limit</td>
</tr>
<tr>
<td>RFC</td>
<td>reference concentration</td>
</tr>
<tr>
<td>RfD</td>
<td>reference dose</td>
</tr>
<tr>
<td>RGD</td>
<td>regional gas dose</td>
</tr>
<tr>
<td>RGDR</td>
<td>regional gas dose ratio</td>
</tr>
<tr>
<td>RR</td>
<td>relative risk</td>
</tr>
<tr>
<td>RT</td>
<td>reverse transcriptase</td>
</tr>
<tr>
<td>RfC</td>
<td>reference concentration</td>
</tr>
<tr>
<td>RfD</td>
<td>reference dose</td>
</tr>
<tr>
<td>SAB</td>
<td>Science Advisory Board</td>
</tr>
<tr>
<td>SCC</td>
<td>squamous cell carcinoma</td>
</tr>
<tr>
<td>SCE</td>
<td>sister chromatid exchange</td>
</tr>
<tr>
<td>SCG</td>
<td>sodium cromoglycate</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SDH</td>
<td>succinate dehydrogenase</td>
</tr>
</tbody>
</table>

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## LIST OF ABBREVIATIONS AND ACRONYMS (continued)

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEER</td>
<td>Surveillance, Epidemiology, and End Results</td>
<td>TL</td>
<td>tail length</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of the mean</td>
<td>TLV</td>
<td>threshold limit value</td>
</tr>
<tr>
<td>SEN</td>
<td>sensitizer</td>
<td>TNF</td>
<td>tumor necrosis factor</td>
</tr>
<tr>
<td>SH</td>
<td>sulfhydryl</td>
<td>TP</td>
<td>total protein</td>
</tr>
<tr>
<td>SHE</td>
<td>Syrian hamster embryo</td>
<td>TRIP</td>
<td>transient receptor potential</td>
</tr>
<tr>
<td>SI</td>
<td>sensory irritation</td>
<td>TRI</td>
<td>Toxic Release Inventory</td>
</tr>
<tr>
<td>SLMA</td>
<td>spontaneous locomotor activity</td>
<td>TWA</td>
<td>time-weighted average</td>
</tr>
<tr>
<td>SMR</td>
<td>standardized mortality ratio</td>
<td>TZCA</td>
<td>thiazolidine-4-carboxylate</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
<td>UCL</td>
<td>upper confidence limit</td>
</tr>
<tr>
<td>SMR</td>
<td>single nucleotide polymorphism</td>
<td>UDS</td>
<td>unscheduled DNA synthesis</td>
</tr>
<tr>
<td>SOD</td>
<td>superoxide dismutase</td>
<td>UF</td>
<td>uncertainty factor</td>
</tr>
<tr>
<td>SOMedA</td>
<td>N⁶-sulfomethyldeoxyadenosine</td>
<td>UFFI</td>
<td>urea formaldehyde foam</td>
</tr>
<tr>
<td>SOMedG</td>
<td>N²-sulfomethyldeoxyguanosine</td>
<td>SSAO</td>
<td>semicarbazide-sensitive amine oxidase</td>
</tr>
<tr>
<td>Sp1</td>
<td>specificity protein</td>
<td>ULLI</td>
<td>unit length labeling index</td>
</tr>
<tr>
<td>SPIR</td>
<td>standardized proportionate incidence ratio</td>
<td>URT</td>
<td>upper respiratory tract</td>
</tr>
<tr>
<td>SSBO</td>
<td>semicarbazide-sensitive amine oxidase</td>
<td>USDA</td>
<td>U.S. Department of Agriculture</td>
</tr>
<tr>
<td>SSB</td>
<td>single strand breaks</td>
<td>VC</td>
<td>vital capacity</td>
</tr>
<tr>
<td>STEL</td>
<td>short-term exposure limit</td>
<td>VOC</td>
<td>volatile organic compound</td>
</tr>
<tr>
<td>TBA</td>
<td>tumor bearing animal</td>
<td>WBC</td>
<td>white blood cell</td>
</tr>
<tr>
<td>TH</td>
<td>T-lymphocyte helper</td>
<td>WDS</td>
<td>wet dog shake</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofolate</td>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>TK</td>
<td>toxicokinetics</td>
<td>WHOROE</td>
<td>World Health Organization Regional Office for Europe</td>
</tr>
</tbody>
</table>

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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic inhalation exposure to formaldehyde. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of formaldehyde.

In Chapter 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, EPA has characterized its overall confidence in the qualitative and quantitative aspects of hazard and dose response by addressing knowledge gaps, uncertainties, quality of data, and scientific controversies. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA’s IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).
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The literature search and preliminary drafts of this document as well as support for editing and formatting were provided by Oak Ridge Institute for Science and Education (ORISE), Oak Ridge Associated Universities (ORAU), Department of Energy, under Interagency Agreement (IAG) Project No. 03-18. The ORISE individuals who contributed to this effort include Sheri Hester, George Holdsworth, Bobette D. Nourse, Wanda Olson, and Lutz W. Weber.

Assistance with the biologically based dose response model evaluation was provided by ENVIRON International Corporation of Monroe, Louisiana (subcontractors to ORISE; Project No. 03-18). The primary scientists involved in the work were Kenny S. Crump and Cynthia Van Ladingham.

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1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of formaldehyde. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m³) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal of entry [POE]) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is a plausible upper bound on the estimate of risk per μg/m³ air breathed.

Development of these hazard identification and dose-response assessments for formaldehyde has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC) (1983). EPA Guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation*
of Biological Values for Use in Risk Assessment (U.S. EPA, 1988), Guidelines for
Developmental Toxicity Risk Assessment (U.S. EPA, 1991), Interim Policy for Particle Size and
Limit Concentration Issues in Inhalation Toxicity (U.S. EPA, 1994a), Methods for Derivation of
Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA,
1994b), Use of the Benchmark Dose Approach in Health Risk Assessment (U.S. EPA, 1995),
Guidelines for Reproductive Toxicity Risk Assessment (U.S. EPA, 1996), Guidelines for
Characterization (U.S. EPA, 2000a), Benchmark Dose Technical Guidance Document (U.S.
EPA, 2000b), Supplementary Guidance for Conducting Health Risk Assessment of Chemical
Mixtures (U.S. EPA, 2000c), A Review of the Reference Dose and Reference Concentration
Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens
(U.S. EPA, 2005b), Science Policy Council Handbook: Peer Review (U.S. EPA, 2006a), and A
Framework for Assessing Health Risks of Environmental Exposures to Children (U.S. EPA,
2006b).

The literature search strategy employed for this compound was based on the Chemical
Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent
scientific information submitted by the public to the IRIS Submission Desk was also considered
in the development of this document. This assessment includes a comprehensive review of
literature through April 2009. As periodic literature searches are conducted by EPA for the
formaldehyde assessment, additional literature identified through December 2009 is included
where that literature was determined to be critical to the assessment. This included a few articles
which were identified through PubMed© searches and publically available as “e-publications” in
2009, but have final publication dates of 2010.
2. BACKGROUND

This chapter provides an overview of the physical and chemical characteristics of formaldehyde. Also provided in this chapter are a description of the production, uses, and sources of formaldehyde and information regarding environmental levels and human exposure. A description of the toxicokinetics and toxicodynamic processes involved in formaldehyde toxicity for the inhalation, oral, and dermal routes can be found in Chapter 3 (Toxicokinetics).

2.1. PHYSICOCHEMICAL PROPERTIES OF FORMALDEHYDE

Formaldehyde (CASRN 50-00-0) is the first of the series of aliphatic aldehydes and is a gas at room temperature. Its molecular structure is depicted in Figure 2-1. It is noted for its reactivity and versatility as a chemical intermediate. It readily undergoes polymerization, is highly flammable, and can form explosive mixtures with air. It decomposes at temperatures above 150°C.

![Figure 2-1. Chemical structure of formaldehyde.](image)

At room temperature, pure formaldehyde is a colorless gas with a strong, pungent, suffocating, and highly irritating odor. Formaldehyde is readily soluble in water, alcohols, ether, and other polar solvents. A synopsis of its physicochemical properties is given in Table 2-1.

2.2. PRODUCTION, USES, AND SOURCES OF FORMALDEHYDE

Formaldehyde has been produced commercially since the early 1900s and, in recent years, has been ranked in the top 25 highest volume chemicals produced in the U.S. (National Toxicology Program [NTP], 2002). In 2003, 4.33 million metric tons of formaldehyde were produced in the U.S. (Global Insight, 2006). In 2000, worldwide formaldehyde production was estimated to be 21.5 million metric tons (International Agency for Research on Cancer [IARC], 2006).

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DRAFT—DO NOT CITE OR QUOTE
Table 2-1. Physicochemical properties of formaldehyde

<table>
<thead>
<tr>
<th><strong>Name</strong></th>
<th><strong>Formaldehyde</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>International Union for Pure and Applied Chemistry name</td>
<td>Formaldehyde</td>
</tr>
<tr>
<td><strong>Synonyms</strong></td>
<td>Formic aldehyde Methanal Methyl aldehyde Methylene oxide Oxomethane Oxymethylene</td>
</tr>
<tr>
<td><strong>Chemical Abstracts Service Index name</strong></td>
<td>Formaldehyde</td>
</tr>
<tr>
<td><strong>Chemical Abstracts Service Registry Number</strong></td>
<td>50-00-0</td>
</tr>
<tr>
<td><strong>Formula</strong></td>
<td>HCHO</td>
</tr>
<tr>
<td><strong>Molecular weight</strong></td>
<td>30.03</td>
</tr>
<tr>
<td><strong>Density</strong>&lt;br&gt;Gas: 1.067 (air = 1)&lt;br&gt;Liquid: 0.815 g/mL at −20°C</td>
<td></td>
</tr>
<tr>
<td><strong>Vapor pressure</strong>&lt;br&gt;3,883 mm Hg at 25°C</td>
<td></td>
</tr>
<tr>
<td><strong>Log K_{ow}</strong></td>
<td>$-0.75$ to $0.35$</td>
</tr>
<tr>
<td><strong>Henry’s law constant</strong>&lt;br&gt;$3.4 \times 10^{-7}$ atm-m$^3$/mol at 25°C&lt;br&gt;$2.2 \times 10^{-2}$ Pa-m$^3$/mol at 25°C</td>
<td></td>
</tr>
<tr>
<td><strong>Conversion factors (25°C, 760 mm Hg)</strong>&lt;br&gt;$1$ ppm = 1.23 mg/m$^3$ (v/v)&lt;br&gt;$1$ mg/m$^3$ = 0.81 ppm (v/v)</td>
<td></td>
</tr>
<tr>
<td><strong>Boiling point</strong>&lt;br&gt;$-19.5$°C at 760 mm Hg</td>
<td></td>
</tr>
<tr>
<td><strong>Melting point</strong>&lt;br&gt;$-92$°C</td>
<td></td>
</tr>
<tr>
<td><strong>Flash point</strong>&lt;br&gt;$60$°C; $83$°C, closed cup for 37%, methanol-free aqueous solution; $50$°C closed cup for 37% aqueous solution with 15% methanol</td>
<td></td>
</tr>
<tr>
<td><strong>Explosive limits</strong></td>
<td>73% upper; 7% lower by volume in air</td>
</tr>
<tr>
<td><strong>Autoignition temperature</strong></td>
<td>$300$°C</td>
</tr>
<tr>
<td><strong>Solubility</strong></td>
<td>Very soluble in water; soluble in alcohols, ether, acetone, benzene</td>
</tr>
<tr>
<td><strong>Reactivity</strong></td>
<td>Reacts with alkalis, acids and oxidizers</td>
</tr>
</tbody>
</table>

Sources: American Conference of Governmental Industrial Hygienists (ACGIH) (2002); International Programme on Chemical Safety (IPCS) (2002); Agency for Toxic Substances and Disease Registry (ATSDR) (1999); Gerberich and Seaman (1994); Walker (1975).
Formaldehyde is a chemical intermediate used in the production of some plywood adhesives, abrasive materials, insulation, foundry binders, brake linings made from phenolic resins, surface coatings, molding compounds, laminates, wood adhesives made from melamine resins, phenolic thermosetting, resin curing agents, explosives made from trimethylolpropane, plumbing components from polyacetal resins, and controlled-release fertilizers made from urea formaldehyde concentrates (IPCS, 1989). Formaldehyde is used in smaller quantities for the preservation and embalming of biological specimens. It is also used as a germicide, an insecticide, and a fungicide in some products. It is found (as an ingredient or impurity) in some cosmetics/personal hygiene products, such as some soaps, shampoos, hair preparations, deodorants, sunscreens, dry skin lotions, and mouthwashes, mascara and other eye makeup, cuticle softeners, nail creams, vaginal deodorants, and shaving cream (IPCS, 2002; ATSDR, 1999).

Formaldehyde is commonly produced as an aqueous solution called formalin, which usually contains about 37% formaldehyde and 12–15% methanol. Methanol is added to formalin to slow polymerization that leads eventually to precipitation as paraformaldehyde. Paraformaldehyde has the formula $(\text{CH}_2\text{O})_n$, where $n$ is 8 to 100. It is essentially a solid form of formaldehyde and therefore has some of the same uses as formaldehyde (Kiernan, 2000). When heated, paraformaldehyde sublimes as formaldehyde gas. This characteristic makes it useful as a fumigant, disinfectant, and fungicide, such as for the decontamination of laboratories, agricultural premises, and barbering equipment. Long-chain polymers (e.g., Delrin plastic) are less inclined to release formaldehyde, but they have a formaldehyde odor and require additives to prevent decomposition (U.S. EPA, 2008).

The major sources of anthropogenic emissions of formaldehyde are motor vehicle exhaust, power plants, manufacturing plants that produce or use formaldehyde or substances that contain formaldehyde (i.e., adhesives), petroleum refineries, coking operations, incineration, wood burning, and tobacco smoke. Among these anthropogenic sources, the greatest volume source of formaldehyde is automotive exhaust from engines not fitted with catalytic converters (NEG, 2003). The Toxic Release Inventory (TRI) data for 2007 show total releases of 21.9 million pounds with about half to the air and half to underground injection (EPA TRI Explorer, http://www.epa.gov/triexplorer/) (U.S. EPA, 2009a).

Formaldehyde is formed in the lower atmosphere by photochemical oxidation of hydrocarbons or other formaldehyde precursors that are released from combustion processes (ATSDR, 1999). Formaldehyde can also be formed by a variety of other natural processes such...
as decomposition of plant residues in the soil, photochemical processes in sea water and forest
fires (National Library of Medicine, 2001).

During smog episodes, indirect production of formaldehyde may be greater than direct
emissions (Fishbein, 1992). Grosjean et al. (1983) estimated the relative contributions of direct
emissions and atmospheric photochemistry to levels of formaldehyde and other carbonyls in Los
Angeles. They found that photochemical production predominates over direct emissions in
controlling formaldehyde levels in Los Angeles air. Using two models, their data were
translated into formaldehyde photochemical production rates of 12–161 tons per day.

Oxidation of methane is the dominant source of formaldehyde in regions remote from
hydrocarbon emissions (Staffelbach et al., 1991). Based on atmospheric measurements at a rural
site in Ontario, Canada and principal component analysis, Li et al. (1994) estimated that
formaldehyde production by atmospheric photochemical oxidation of hydrocarbons is
approximately 16 times that from primary emissions.

The input of formaldehyde into the environment is counterbalanced by its removal by
several pathways. Formaldehyde is removed from the air by direct photolysis and oxidation by
photochemically produced hydroxyl and nitrate radicals. Measured or estimated half-lives for
formaldehyde in the atmosphere range from 1.6 to 19 hours, depending upon estimates of radiant
energy, the presence and concentrations of other pollutants, and other factors (ATSDR, 1999).
Given the generally short daytime residence times for formaldehyde, there is limited potential for
long-range transport (IPCS, 2002). In cases where organic precursors are transported long
distances, however, secondary formation of formaldehyde may occur far from the anthropogenic
sources of the precursors.

Formaldehyde is released to water from the discharges of both treated and untreated
industrial wastewater from its production and from its use in the manufacture of formaldehyde-
containing resins (ATSDR, 1999). Formaldehyde is also a possible drinking-water disinfection
by-product from the use of ozone and/or hydrogen peroxide. In water, formaldehyde is rapidly
hydrated to form a glycol, and the equilibrium favors the glycol.

2.3. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

General population exposure to formaldehyde can occur via inhalation, ingestion and
dermal contact. Each of these pathways and associated media levels are discussed below.
Formaldehyde exposure can also occur occupationally via three main scenarios:
The production of aqueous solutions of formaldehyde (formalin) and their use in the chemical industry (e.g., for the synthesis of various resins, as a preservative in medical laboratories and embalming fluids, and as a disinfectant).

Release from formaldehyde-based resins in which it is present as a residue and/or through their hydrolysis and decomposition by heat (e.g., during the manufacture of wood products, textiles, synthetic vitreous insulation products, and plastics). In general, the use of phenol-formaldehyde resins results in much lower emissions of formaldehyde than those of urea-based resins.

The pyrolysis or combustion of organic matter (e.g., in engine exhaust gases or during firefighting) (IARC, 2006).

Industries with the greatest potential for exposure include health services, business services, printing and publishing, manufacture of chemicals and allied products, manufacture of apparel and allied products, manufacture of paper and allied products, personal services, machinery (except clerical), transport equipment, and furniture and fixtures (IARC, 1995).

2.3.1. Inhilation

The most current ambient air monitoring data for formaldehyde come from EPA’s air quality system database (EPA’s AirData Web site: http://www.epa.gov/air/data/index.html) (U.S. EPA, 2009b). These data have been collected from a wide variety of sources, including state and local environmental agencies, but have not been collected from a statistically based survey. The most recent data, for the year 2007, come from 188 monitors located in 33 states as shown in Figure 2-2 (U.S. EPA, 2008). The annual means for these monitors range from 0.7–45.03 μg/m$^3$ (0.56–36.31 ppb) and have an overall average of 3.44 μg/m$^3$ (2.77 ppb). The annual means are derived by EPA by averaging all available daily data from each monitor. Table 2-2 shows a breakout of the data by land use category based on the annual means from each monitor for 2005, 2006, and 2007. The land use is established on the basis of the most prevalent land use within 0.25 miles of the monitor. The mobile category (land near major highways or interstates such that it is primarily impacted by mobile sources) has the highest mean levels, and agricultural lands have the lowest.

Under the National-Scale Air Toxics Assessment (NATA) program, EPA has conducted an emissions inventory for a variety of hazardous air pollutants (HAPs), including formaldehyde (U.S. EPA, 2006c). The NATA uses the emissions inventory data to model nationwide air concentrations/exposures (U.S. EPA, 2006c). The results of the 1999 ambient air concentration modeling for formaldehyde suggest that county median air levels range from 0 to 6.94 μg/m$^3$ (0–5.59 ppb) with a national median of 0.56 μg/m$^3$ (0.45 ppb) (see Figure 2-3). Similar results
Figure 2-2. Locations of hazardous air pollutant monitors. Dasgupta et al. (2005) measured formaldehyde levels in 5 U.S. cities during 1999–2002. Samples were collected over approximately a one month period in the spring or summer. Mean levels were 5.05 ppb in Nashville, TN; 7.96 ppb in Atlanta, GA; 4.49 ppb in Houston, TX; 3.12 ppb in Philadelphia, PA; and 2.63 in Sydney, FL.

Table 2-2. Ambient air levels by land use category

<table>
<thead>
<tr>
<th>Formaldehyde exposure by category&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Agriculture</th>
<th>Commercial</th>
<th>Forest</th>
<th>Industrial</th>
<th>Mobile&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Residential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of data points</td>
<td>17</td>
<td>166</td>
<td>19</td>
<td>61</td>
<td>16</td>
<td>282</td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>2.08 ± 0.98</td>
<td>3.26 ± 2.76</td>
<td>2.79 ± 2.17</td>
<td>6.28 ± 14.45</td>
<td>6.84 ± 7.28</td>
<td>2.75 ± 1.71</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.34</td>
<td>0.20</td>
<td>0.40</td>
<td>0.14</td>
<td>2.02</td>
<td>0.17</td>
</tr>
<tr>
<td>Maximum</td>
<td>4.34</td>
<td>20.61</td>
<td>7.33</td>
<td>74.72</td>
<td>23.39</td>
<td>12.35</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are µg/m³.

<sup>b</sup>“Mobile” is ambient air in locations primarily impacted by mobile sources.

Figure 2-3. Modeled ambient air concentrations based on 1999 emissions.

were found for the year 2002: county concentrations ranged from 0.12 to 9.17 µg/m³ (0.097–7.38 ppb) with median of 0.78 µg/m³ (0.63 ppb). NATA has not provided updated concentration maps for 2002. The 1999 map shows the highest levels in the far west and northeastern regions of the U.S. While these modeling results can be useful, it is important to consider their limitations. Some of the geographical differences result from differences in methods used by states supplying the data. For example, the high levels indicated for Idaho result from the large amount of wood burned during forest fires and the relatively high emission factor that Idaho uses (compared with other states) to estimate formaldehyde emissions from forest fires. A comparison of modeling results from NATA to measured values at the same locations is presented in EPA (2006c). For 1999, it was found that formaldehyde levels were underestimated at 76% of the sites (n = 68). One possible reason why the NATA results appear low compared to measurements is that the modeling has not accounted for secondary formation of formaldehyde in the atmosphere.

In general, ambient levels of formaldehyde in outdoor air are significantly lower than those measured in the indoor air of workplaces or residences (ATSDR, 1999; IARC, 1995).

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Indoor sources of formaldehyde in air include volatilization from pressed wood products, carpets, fabrics, insulation, permanent press clothing, latex paint, and paper bags, along with emissions from gas burners, kerosene heaters, and cigarettes (NLM, 2001). In general, the major indoor air sources of formaldehyde can be described in two ways: (1) those sources that have the highest emissions when the product is new with decreasing emission over time, as with the first set in the examples above; and (2) those sources that are reoccurring or frequent such as the second set of examples above. Gilbert et al. (2006) studied 96 homes in Quebec City, Canada and found elevated levels in homes with new wood or melamine furniture purchased within the previous 12 months. A summary of indoor data is provided in Table 2-3. Results vary depending on housing characteristics and date of study.

Salthammer et al. (2010) present a thorough review of formaldehyde sources and levels found in the indoor environment. Based on an examination of international studies carried out in 2005 or later they conclude that the average exposure of the population to formaldehyde is 20 to 40 μg/m³ under normal living conditions. They used the diagram shown in Figure 2-4 to summarize data they found on the range of formaldehyde air concentrations (in ppb) in different environments.

Data on formaldehyde levels in outdoor and indoor air were collected under Canada’s National Air Pollution Surveillance program (IPCS, 2002; Health Canada and Environment Canada, 2001). The effort included four suburban and four urban sites sampled in the period 1990–1998. A Monte Carlo analysis applied to the pooled data (n = 151) was used to estimate the distribution of time-weighted 24-hour air exposures. This study suggested that mean levels in outdoor air were 3.3 μg/m³ (2.7 ppb) and mean levels in indoor air were 35.9 μg/m³ (29.2 ppb) (Health Canada and Environment Canada, 2001). The simulation analysis also suggested that general population exposures averaged 33–36 μg/m³ (27–30 ppb).

Since the early to mid 1980s, manufacturing processes and construction practices have been changed to reduce levels of indoor formaldehyde emissions (ATSDR, 1999). A 2008 law enacted by the California Air Resource Board (CARB. 2008, Final Regulation Order: Airborne Toxic Control Measure to Reduce Formaldehyde Emissions from Composite Wood Products; http://www.arb.ca.gov/regact/2007/compwood07/fro-final.pdf) has limited the amount of formaldehyde that can be released by specific composite wood products (i.e., hardwood plywood, particle board, and medium density fiberboard) sold, supplied, or manufactured for use in California. For this reason the mean indoor air levels presented by Health Canada and Environment Canada (2001) (based on samples collected from 1989–1995) may overestimate
### Table 2-3. Studies on residential indoor air levels of formaldehyde (nonoccupational)

<table>
<thead>
<tr>
<th>Citation</th>
<th>No. of samples</th>
<th>Target population/house type</th>
<th>Mean ($\mu$g/m$^3$)</th>
<th>Range ($\mu$g/m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold et al., 1993</td>
<td></td>
<td>Complaint homes$^1$</td>
<td>&lt;60</td>
<td>24–960</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Older conventional homes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hare et al., 1996</td>
<td></td>
<td>Newly built homes</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>Hare et al., 1996</td>
<td></td>
<td>30 days after installing pressed wood</td>
<td>42–540</td>
<td></td>
</tr>
<tr>
<td>Gammage and Hawthorne, 1985</td>
<td>&gt;1,200, 131, 500</td>
<td>Homes with UFFI</td>
<td>60–144</td>
<td>12–4080</td>
</tr>
<tr>
<td></td>
<td>260</td>
<td>Homes without UFFI</td>
<td>30–84</td>
<td>12–204</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Complaint mobile homes</td>
<td>120–1080</td>
<td>0–5040</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Newer mobile homes</td>
<td>1032</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Older mobile homes</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>Hawthorne et al., 1986a, b</td>
<td>18, 11, 11, 40</td>
<td>Conventional homes 0–5 yr</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Conventional homes 5–15 yr</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Conventional homes &gt;15 yr</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Conventional homes overall</td>
<td>72</td>
<td>24–480</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Noncomplaint, mobile homes, randomly selected</td>
<td>109–744</td>
<td>12–3480</td>
</tr>
<tr>
<td>Health Canada and Environment Canada, 2001</td>
<td>151</td>
<td>Residential (Canadian) noncomplaint homes</td>
<td>35</td>
<td>?–148</td>
</tr>
<tr>
<td>Zhang et al., 1994a, b</td>
<td>6</td>
<td>Residential, carpeted, nonsmoking homes</td>
<td>66</td>
<td>42–89</td>
</tr>
<tr>
<td>Gilbert et al., 2006</td>
<td>96</td>
<td>Residential (Canadian)</td>
<td>29.5</td>
<td>9.6–90.0</td>
</tr>
<tr>
<td>Shah and Singh, 1988</td>
<td>315</td>
<td>Residential and commercial</td>
<td>59</td>
<td>23–89</td>
</tr>
<tr>
<td>Stock, 1987</td>
<td>43</td>
<td>Conventional homes</td>
<td>84</td>
<td>96–216</td>
</tr>
<tr>
<td>Krzyzanowski et al., 1990</td>
<td>202</td>
<td>Conventional homes</td>
<td>31</td>
<td></td>
</tr>
</tbody>
</table>

$^1$ The "complaint" homes are ones where the occupants have complained about formaldehyde irritant symptoms.

Note: 1 ppb = 1.2 $\mu$g/m$^3$. 

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current levels. In addition, the Canadian indoor air data may overestimate formaldehyde levels in U.S. homes, because many residential homes in Canada use wood burning stoves more frequently and have tighter construction (due to colder winters), leading to less dilution of indoor emissions. The outdoor air levels, however, appear to have remained fairly constant over recent years, and the median outdoor level from the Canadian study (2.8 µg/m³) (2.3 ppb) is very similar to the median of the U.S. monitoring data (2.83 µg/m³) (2.3 ppb) in 1999.

Even though formaldehyde levels in construction materials have declined, indoor inhalation concerns still persist. For example, recent studies have measured formaldehyde levels in mobile homes/trailers (these terms are used interchangeably here to refer to homes with wheels that are designed to be moved). ATSDR (2007) reported on air sampling in 96 unoccupied trailers provided by the Federal Emergency Management Agency (FEMA) used as temporary housing for people displaced by Hurricane Katrina. Formaldehyde levels in closed trailers averaged 1,250 ± 828 µg/m³ (mean ± standard deviation [SD]) (1.04 ± 0.69 ppm), with a range of 12–4,390 µg/m³ (0.01–3.66 ppm). The levels decreased to an average of 468 ± 31 µg/m³ (0.39 ± 0.26 ppm) in 1999.

Source: Salthammer et al. (2010).

Figure 2-4. Range of formaldehyde air concentrations (ppb) in different environments.
324 µg/m³ (0.39 ± 0.27 ppm), with a range of 0.00–1,960 µg/m³ (0.00–1.63 ppm) when the air conditioning was turned on. Levels also decreased to an average of 108 ± 96 µg/m³ (0.09 ± 0.08 ppm), with a range of 12–588 µg/m³ (0.01–0.49 ppm) when the windows were opened. ATSDR (2007) found an association between temperature and formaldehyde levels; higher temperatures were associated with higher formaldehyde levels in trailers with the windows closed. They also noted that different commercial brands of trailers yielded different formaldehyde levels.

In December 2007 and January 2008, the Centers for Disease Control and Prevention (CDC) measured formaldehyde levels in a stratified random sample of 519 FEMA-supplied occupied travel trailers, park models, and mobile homes (“trailers”) (CDC, 2008). At the time of the study, sampled trailers were in use as temporary shelters for Louisiana and Mississippi residents displaced by hurricanes Katrina and Rita. The geometric mean level of formaldehyde in sampled trailers was 95 µg/m³ (77 ppb), and the range was 3.7–730 µg/m³ (3–590 ppb).

2.3.2. Ingestion

Limited U.S. data indicate that concentrations in drinking water may range up to approximately 10 µg/L in the absence of specific contributions from the formation of formaldehyde by ozonation during water treatment or from leaching of formaldehyde from polyacetyl plumbing fixtures (IPCS, 2002). In the absence of other data, one-half this concentration (5 µg/L) was judged to be a reasonable estimate of the average formaldehyde in Canadian drinking water. Concentrations approaching 100 µg/L were observed in a U.S. study assessing the leaching of formaldehyde from domestic polyacetal plumbing fixtures, and this concentration was assumed to be representative of a reasonable worst case (IPCS, 2002).

Formaldehyde is a natural component of a variety of foodstuffs (IARC, 1995; IPCS, 1989). However, foods may be contaminated with formaldehyde as a result of fumigation (e.g., grain fumigation), cooking (as a combustion product), and release from formaldehyde resin-based tableware (IARC, 1995). Also, the compound has been used as a bacteriostatic agent in some foods, such as cheese (IARC, 1995). There have been no systematic investigations of levels of formaldehyde in a range of foodstuffs that could serve as a basis for estimation of population exposure (Health Canada and Environment Canada, 2001). According to the limited available data, concentrations of formaldehyde in food are highly variable. In the few studies of the formaldehyde content of foods in Canada, the concentrations were within a range of <0.03–14 mg/kg (Health Canada and Environment Canada, 2001). Data on formaldehyde levels in food have been presented by Feron et al. (1991) and IPCS (1989) from a variety of studies, yielding the following ranges of measured values:

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• Fruits and vegetables: 3–60 mg/kg
• Meat and fish: 6–20 mg/kg
• Shellfish: 1–100 mg/kg
• Milk and milk products: 1–3.3 mg/kg

Daily intake of formaldehyde was estimated by IPCS (1989) to be in the range of 1.5–14 mg for an average adult. Similarly, Fishbein (1992) estimated that the intake of formaldehyde from food is 1–10 mg/day but discounted this on the belief that it is not available in free form. Although the bioavailability of formaldehyde from the ingestion of food is not known, it is not expected to be significant (ATSDR, 1999). Using U.S. Department of Agriculture (USDA) (1979) consumption rate data for various food groups, Owen et al. (1990) calculated that annual consumption of dietary formaldehyde results in an intake of about 4,000 mg or approximately 11 mg/day.

2.3.3. Dermal Contact

The general population may have dermal contact with formaldehyde-containing materials, such as some building products and cosmetics (see Section 2.2 for the details on these products). Generally, though, dermal contact is more of a concern in occupations that involve handling concentrated forms of formaldehyde, such as those occurring in embalming and chemical production.
3. TOXICOKINETICS

This chapter presents chemical specific information on the toxicokinetics of formaldehyde which helps to inform the potential for health effects from formaldehyde exposure. As a water soluble and reactive gas (see Chapter 2), the chemical reactions of formaldehyde at the site of first contact in biological systems is important to understanding its toxic potential. Therefore, before a discussion of the absorption, distribution, and metabolism of formaldehyde (which normally comprises the heart of the toxicokinetic discussion of an agent) a section is provided which discusses some key issues regarding formaldehyde’s reactivity. Section 3.1 provides information regarding the hydration of formaldehyde in biological aqueous systems and the equilibrium which exists between free formaldehyde and methylene glycol. Additional information is provided on what is known of the nature of chemical reactions of free formaldehyde with proteins. These discussions are provided to give context to the following Sections of Chapter 3.

Sections 3.2 and 3.3 present the available studies which describe the absorption and distribution of formaldehyde, including animal studies of radiolabeled formaldehyde. The influence of formaldehyde’s reactivity at the site of first contact and effects on the mucociliary apparatus are presented here as well, as these effects may modify the uptake of formaldehyde. Metabolism of formaldehyde is presented in Section 3.4, but the endogenous production of formaldehyde from normal metabolic processed, as well as metabolism of other xenobiotics. The last section of Chapter 3 present the available models which apply to the toxicokinetics of formaldehyde—in this case primarily modeling of the flux of formaldehyde through tissues at the sight of first contact using computational fluid dynamics models.

3.1. CHEMICAL PROPERTIES AND REACTIVITY

Formaldehyde (HCHO) is the smallest aldehyde (30 g/mol) and is a gas at room temperature. It is highly water soluble and reactive. In water, less than 0.1% of formaldehyde exists unhydrated, with the majority reported to be in the hydrated form, methylene glycol (CH$_2$(OH)$_2$) (Priha et al., 1996). Formaldehyde reacts readily with high and low molecular weight biological constituents.

3.1.1. Hydration of Formaldehyde

In aqueous solution formaldehyde exists in equilibrium with its hydrated form methanediol (CH$_2$OH$_2$) ($K_d = 5.5 \times 10^{-4}$). The equilibrium favors methanediol at physiological
temperature and pH (>99.9%) and is readily reversible. In biological systems, as free formaldehyde is removed from aqueous solution through binding with serum proteins and cellular components, the equilibrium is reestablished by dehydration of methanediol to free formaldehyde. The reversible nature of this hydration reaction describes how a pool of free formaldehyde may be sustained in biological systems.

3.1.2. Binding of Formaldehyde to Proteins

Formaldehyde is a reactive molecule that is likely to react with both low molecular weight cellular components (e.g., reduced glutathione[GSH]) as well as high molecular weight components. Unlike deoxyribonucleic acid (DNA), which has some additional barriers to exposure (i.e., nucleus), extracellular and intracellular proteins are obvious targets for interacting with formaldehyde. Formaldehyde is a well-known cross-linking agent that is used in the fixation of tissues, preparation of vaccines, and study of protein-protein interactions (Metz et al., 2006). However, the exact nature of the protein modifications used for these purposes is not yet fully characterized (Metz et al., 2006, 2004). Figure 3-1 provides a general reaction scheme for formaldehyde-mediated modifications of amino acids. In step 1, formaldehyde reacts with primary N-terminal amines to form a labile methylol adduct. This adduct can undergo dehydration (step 2) to form an imine, or Schiff base (−N=CH2). Metz et al. (2004) examined the types of formaldehyde-protein reactions that are likely to occur in vivo by synthesizing several identical polypeptides with one varying amino acid (X) within the sequence VELXVLL (V = valine, E = glutamate, L = Leucine, X = varying amino acid). Several peptides with reactive amino acids did not exhibit modifications, suggesting that the peptide sequence/structure affects the ability of formaldehyde to react with amino acids. Peptides that were modified indicated formation of methylol adducts (see Figure 3-1, step 1) or a mixture of methylol and imine adducts (see Figure 3-1, step 2).

Mucus is composed of water, electrolytes, polysaccharides, and about 0.5% soluble proteins (Priha et al., 1996; Bogdanffy et al., 1987). Bogdanffy et al. (1987) showed that although human nasal mucus can bind 70% of 100 mM formaldehyde, irreversible binding of [14C]-formaldehyde to serum albumin (the major protein in mucus) was shown to be insignificant after a 1-hour incubation. Irreversible binding (50% or more) did not occur until after about 7 hours of incubation. These data suggest that the protein content of mucus may not provide a significant formaldehyde irreversible sink. Nonetheless, the solubility of formaldehyde in mucus along with mucus flow and ingestion likely indicate that much of the inhaled dose is removed—perhaps as much as 42% in rodents (IARC, 2005; Schlosser, 1999).
Note: Formaldehyde reacts with primary N-terminal amines to form a methylol adduct [1], which increases the molecular weight by 30 Da (Δm). This labile adduct can rearrange to form an amine, or Schiff base [2], that results in an increase in MW of 12 Da. Schiff bases can react with certain amino acids to form intra- or intermolecular methylene bridges [3]. The two amino acids depicted in step 3 may be within the same protein or possibly from two different proteins.

In general, formaldehyde interacts with proteins. Studies carried out in cell culture media containing serum and formaldehyde have shown that such mixtures are quite labile. For example, during a 60-minute incubation of formaldehyde with complete cell media (i.e., with fetal calf serum) at 38°C, gas chromatography-mass spectrometry (GC-MS) exhibited very different peak profiles at different points during the incubation (Proctor et al., 1986). In contrast, GC-MS chromatograms of cell media containing formaldehyde but no serum proteins appeared relatively unchanged throughout the incubation. Compared to cell culture medium alone, complete media were considered to provide a more suitable model for the hypothetical interactions that formaldehyde could undergo in vivo (including perhaps blood).

Source: Metz et al. (2004).
3.2. ABSORPTION

3.2.1. Oral

Oral absorption of [14C]-formaldehyde (7 mg/kg) in rats resulted in 40% elimination as 14C-carbon dioxide (14CO2), with 10% excretion in urine, 1% excretion in feces, and much of the remaining 49% retained within the carcass, presumably due to metabolic incorporation (IARC, 1995; Buss et al., 1964).

3.2.2. Dermal

Jeffcoat et al. (1983) reported on the disposition of various doses of [14C]-formaldehyde dermally administered to rats, guinea pigs, and monkeys. Very little (<1% of the applied dose) of the radiolabel was found in the major organs excised during necropsy. As noted by the authors, the disposition of formaldehyde when administered via the dermal route was markedly different to that observed when the compound was administered intravenously or intraperitoneally. In the latter cases, there was much evidence of metabolic activity, and substantial portions of the load were expired as CO2. The difference appeared to be the result of a reaction of dermally applied formaldehyde with macromolecules at or near the skin surface or of its evaporation. In general, portions of the load that succeed in entering the circulation probably do so bound to macromolecules or by incorporation of the radiolabel via the one-carbon pool. Likewise, Bartnik et al. (1985) who applied [14C]-formaldehyde to the shaved backs of rats concluded that the overwhelming majority of the formaldehyde load remained sequestered in the outer layers of skin at or near the site of application. At the end of the various measurements, approximately 70% of the dose was found in the treated skin, with a marked localization of the remaining radioactivity in the uppermost layers. This fraction of the load was considered to be permanently sequestered, most likely as a result of irreversible binding to macromolecular components.

3.2.3. Inhalation

Studies indicate that the majority of inhaled formaldehyde is absorbed in the upper respiratory tract (URT) but that the extent of the scrubbing in this region varies significantly across species. In dogs, nearly 100% of nasally inhaled formaldehyde is absorbed (Egle, 1972). Lower respiratory tract (LRT) studies designed to collect formaldehyde via a tube inserted into the lower trachea revealed that nearly 95% of formaldehyde was absorbed during the first pass through the upper respiratory tract (Egle, 1972), an effect observed with multiple ventilation rates. The rat nasal passages also scrub nearly all of the inhaled formaldehyde (on average ~97%) (Morgan et al., 1986). In computational dosimetry modeling based on anatomically...
realistic representation of the human nasal airways from a single individual, approximately 90% of inhaled formaldehyde was predicted to be absorbed in the nose at resting inspiration. As the inspiratory rate increased, this fraction decreased to about 70% at light exercise and to 58% at heavy exercise conditions (see Figure 1 in Kimbell et al. [2001b]). The normal human breathing mode during heavy exercise is oronasal (with ~54% of airflow being oral) (ICRP 66, 1994). Consequently, it is estimated that during heavy exercise breathing (50 L/minute) the flux of formaldehyde into tissue (or rate of mass transported per mm² of tissue surface area) in the first six to eight generations of the tracheobronchial airways is comparable to that in the nasal region (Overton et al., 2001).

It is important to note that the computer simulations mentioned above are based on anatomical representations of a single individual. Significant anatomical variations occur in human nasal airways. For example, the nasal volumes of 10 adult nonsmoking subjects between 18 and 50 years of age in a study in the U.S. varied between 15 and 60 mL (Santiago et al., 2001), and disease states can result in considerable further variation (Singh et al., 1998).

Species differences in kinetic factors have been argued to be the key determinants of species-specific lesion distributions for formaldehyde and other reactive inhaled gases. Airway geometry is an important determinant of inhaled-formaldehyde dosimetry in the respiratory tract and its differences across species. These issues will be discussed in a later section on dosimetry modeling.

### 3.2.3.1. Formaldehyde Uptake Can Be Affected by Effects at the Portal of Entry

Certain formaldehyde-related effects have the potential to modulate its uptake and clearance. The mucociliary apparatus of the upper respiratory tract is the first line of defense against airborne toxins. Comprising a thick mucus layer (epiphase), hydrophase, and a ciliated epithelium, the mucociliary apparatus may entrain, neutralize, and remove particulates and airborne chemicals from inspired air. As reviewed by Wolfe (1986), airborne pollutants and reactive gases have been shown to decrease mucus flow rates in several animal models (Mannix et al., 1983; Iravani, 1974; Carson et al., 1966; Dalhamn, 1956; Cralley, 1942). Degradation in the continuity or function of this mucociliary apparatus could result in a lower clearance of inhaled pollutants at the portal of entry.

Morgan et al. (1983) first reported defects in mucociliary function in F344 rats exposed to 15 ppm formaldehyde 6 hours/day for 1–9 days. Mucostasis occurred in several regions in all rats after a single 15 ppm exposure. Ciliastasis occurred with greater frequency and across more regions of the nasoturbinate in subsequent days of exposure. The authors observed that mucostasis preceded ciliastasis in most cases, and vigorous ciliary activity was noted in areas

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without mucus flow. Morgan et al. (1984a) also studied formaldehyde effects on the mucociliary apparatus of isolated frog palates in vitro. Mucostasis was evident as mucus became stiff and eventually rigid with increasing formaldehyde concentration and time of exposure. Ciliary beat continued even after mucostasis, but ciliastasis ultimately occurred when exposure reached 4 and 9 ppm.

When a rodent is exposed to an irritant, its inhaled dose and pattern of deposition can be profoundly affected by reflex bradypnea, a protective reflex seen in rodents but not in humans. Reflex bradypnea can occur when the trigeminal nerve is exposed to a sufficient concentration of an irritant, such as formaldehyde. It is manifest as markedly decreased activity or prostration, reduced metabolism, hypothermia (as much as 5°C), significantly reduced respiratory rate and minute volume, and altered blood and brain chemistry. Because of their small size, rodents are able to rapidly lower their metabolism and body temperature and therefore their oxygen demand. The consequence is that their inhaled dose of an irritating chemical is dramatically lowered.

Reflex bradypnea is quantified as the RD<sub>50</sub>, which is the concentration of a chemical that results in a 50% decrease in respiratory rate. It can take as much as two hours for rodents to fully recover from the effects of reflex bradypnea. The clinical manifestations of reflex bradypnea can easily be misconstrued as toxicity. None of the studies described in this assessment took into account the fact that reflex bradypnea may have confounded the results. Reflex bradypnea is discussed in depth in Section 4.2.1.1.

Sensory irritation studies suggest that formaldehyde activates the trigeminal nerve by activating nociceptors through the modification of receptor amino acids, possibly including thiol groups. Cassee et al. (1996) measured sensory irritation to formaldehyde, acetaldehyde, and acrolein in male Wistar rats, following a 30-minute nose-only exposure. Formaldehyde and acrolein elicited similar responses, whereas acetaldehyde was far less irritating. The authors suggested that the differences in sensitivity to the aldehydes might be explained by differences in physicochemical properties and by regional differences in activities of detoxifying enzymes for each chemical. In addition, it has been suggested that acetaldehyde might interact with sensory nerves via an amino group (Steinhagen and Barrow, 1984), whereas the receptor-binding site for formaldehyde and acrolein is believed to be a thiol group. Differential binding sites for sensory irritants in the trigeminal nerve have been reported (Nielsen, 1991).

Sensory irritation effects are discussed in depth in Chapter 4 but are noted here because stimulation of the trigeminal nerve by formaldehyde can result in significantly lower pulmonary ventilation, and formaldehyde exposure in rodents at concentrations that approach the RD<sub>50</sub>. Barrow et al. (1983) have estimated the “inhaled dose” equivalent to an exposure concentration of 15 ppm in mice and rats used in the chronic formaldehyde bioassays by Kerns et al. (1983)
and Monticello and Morgan (1994). Their results indicate that, because mice are observed to
decrease their minute volume by approximately 75% as compared to 45% in rats, a twofold
greater inhaled dose would be expected in rats versus mice. This difference may be relevant to
the increased incidence of squamous cell carcinoma of the nasal cavity in F344 rats as compared
to B6C3F1 mice. Chang et al. (1983) estimated a reduction of 25% in the minute volume of
F344 rats. Yokley et al. (2008) have recently published a model that accounts for physiological
changes in ventilation rate induced by sensory irritation in rats. Thus, the “standard” minute
volumes used for rats and mice need to be adjusted downward when calculating dosimetric
adjustment factors for extrapolation of adverse effects to humans (Thompson et al., 2008). This
question is further discussed in the section on modeling the dosimetry.

Another effect that modulates dosimetry is the dynamic tissue remodeling of nasal
airways that occurs as a consequence of exposure to reactive gases. For example, formaldehyde
dosimetry is influenced by the occurrence of squamous metaplasia, an adaptive tissue conversion
to squamous that occurs in nasal epithelium exposed to toxic levels of formaldehyde. The
metaplasia has been observed to occur in rats at exposure concentrations of 3 ppm and higher
(Kimbell et al., 1997b). Squamous epithelium is known to absorb considerably less
formaldehyde than other epithelial types (Kimbell et al., 1997b). Overall, the highest flux levels
of formaldehyde in the simulations of the rat nose in Kimbell et al. (2001a) are estimated in the
region just posterior to the nasal vestibule. A consequence of squamous metaplasia would be to
“push” the higher levels of formaldehyde flux toward the more distal regions of the nose
(Kimbell et al., 1997b). Subramaniam et al. (2008) discussed this issue further in the context of
uncertainties in the modeling of formaldehyde dosimetry.

3.3. DISTRIBUTION

3.3.1. Transport of Methylene Glycol

In biological systems, formaldehyde is known to exist in equilibrium with its hydrated
form, as methanediol (CH₂OH₂) (Kᵣ = 5.5 × 10⁻⁴) at physiological temperatures and pH
(>99.9%) in the body and is readily reversible. When free formaldehyde is removed from
aqueous solution through binding with serum proteins and cellular components, the equilibrium
is reestablished by dehydration of methanediol to free formaldehyde. Thus, a pool of free
formaldehyde may be sustained in biological systems due to the reversible nature of this
hydration reaction.

There is strong and consistent evidence in biological testing systems in vitro that treating
cells with formaldehyde in an aqueous media results in significant cytotoxicity, cell proliferation,
clastogenic effects and clear evidence of mutational events (see Section 4.3). Similarly, animal
bioassays where formaldehyde is administered in drinking water report portal of entry toxicity including hyperplasia, increased cell proliferation, focal lesions and tumors (see Section 4.2.1). It should be noted that URT tissues are covered by an aqueous mucous layer, through which formaldehyde must pass to react the cellular components of the URT. It has been postulated that formaldehyde transports through this mucous layer and the underlying tissues as methanediol (Georgieva et al., 2003).

The dynamic equilibrium between the hydrated and unhydrated forms of formaldehyde in biological systems is well understood. Since the hydration reaction favors methanediol, it is expected that exogenous formaldehyde which reaches the blood will primarily exist as methanediol and is subject to physiological elimination. As free, unhydrated formaldehyde continues to react with serum proteins and cellular components, the blood levels of methanediol are expected to reduce as it is dehydrated to maintain equilibrium. Although some attempts to measure significant changes in free formaldehyde levels in blood after inhalation exposure have not been successful, the half-life in blood has been measured after i.v. injection at approximately 2 minutes (McMartin et al., 1979). Additionally, the detection of antibodies to formaldehyde-hemoglobin adducts and formaldehyde-albumin adducts in exposures workers, smokers and laboratory animals exposed via inhalation provides direct evidence that formaldehyde is able to react with serum albumin and hemoglobin in biological systems (Li et al., 2007; Varro et al., 1997; Grammer et al., 1993; Dykewicz et al., 1991; Thrasher et al., 1990, Grammer et al., 1990). These data support the hypothesis that exogenous formaldehyde may reach and transport through the blood. If so, formaldehyde (or methanediol) may reach sites distal to the portal of entry.

3.3.2. Formaldehyde-GSH Conjugate as a Method of Systemic Distribution

Formaldehyde is primarily metabolized by alcohol dehydrogenase (ADH3) which uses the formaldehyde-glutathione hemiacetal adduct as the substrate. Sanghani et al. (2000) have shown that due to high circulating concentrations (50-fold) of glutathione in human blood, the S-(hydroxymethyl)glutathione (HMGSH) adduct, the nonenzymatic product of formaldehyde with glutathione is the major form of formaldehyde seen in vivo (Sanghani et al., 2000). It is likely that the reversibly bound HMGSH may be transported to different tissues through circulation, but, specific experimental evidence is lacking.

3.3.3. Levels in Blood

Inhalation studies in several species indicate that exposure to formaldehyde does not result in elevated levels in blood. These studies were carried out over a wide range of exposure concentrations and durations. Rats exposed to 14 ppm formaldehyde for 2 hours exhibited no
increase in blood formaldehyde levels [2.25 ± 0.07 µg/(g blood) in treated animals compared with 2.24 ± 0.07 µg/(g blood) in control animals] when measured by GC-MS using a stable isotope dilution technique (Heck et al., 1985, 1982). Similarly, mean formaldehyde blood levels in humans (n = 6) exposed to 1.9 ppm formaldehyde for 40 minutes in a walk-in chamber (2.77 ± 0.28 µg/g blood) were not statistically different from measurements in the same population before exposure (mean of 2.61 ± 0.14 µg/g) (Heck and Casanova-Schmitz, 1984). The variability in the levels was large. At the individual level, the data showed both increase and decrease in blood levels relative to pre-exposure levels, which was attributed by the authors as plausibly due to temporal variations in baseline levels in humans, particularly since the experiment did not control food intake prior to exposure. Studies in rhesus monkeys have revealed endogenous formaldehyde levels (2.4 µg/g blood) comparable to humans and that levels were also unaltered following exposure to 6 ppm formaldehyde via inhalation 6 hours/day for 4 weeks, measurements being taken at both 7 minutes and 45 hours post final exposure (Casanova et al., 1988).

It is important to keep in mind that the GC-MS method is not capable of detecting irreversibly bound formaldehyde; for example, formaldehyde levels detected by this method, even in the anterior nasal mucosa of rats exposed to 6 ppm of formaldehyde, were not elevated over control levels. Furthermore, the GC-MS method does not differentiate between free and reversibly bound adducts of formaldehyde (Heck et al., 1982). Thus, measured levels represent total formaldehyde concentration that includes free formaldehyde as well as reversibly bound adducts. Based on the known Michaelis-Menten constant, Km, for formaldehyde dehydrogenase with respect to the GSH adduct formation, Heck et al. (1982) estimated under certain assumptions that free formaldehyde comprised only about 1–2% of the total formaldehyde measured by their method. Furthermore, as shown by Metz et al. (2006, 2004), formaldehyde reactions with primary amino and thiol groups can, in a second step, react with many other amino acids to form stable methylene bridges. Presumably, such reactions would not be detectable by using the methods employed by Heck et al. (1982).4 Thus, the limited interpretation of GC-MS measurements of blood levels suggests that formaldehyde does not appreciably reach the blood.

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4 Additionally, note that, although Heck et al. (1982) demonstrated that formaldehyde concentration can be accurately measured from glutathione and tetrahydrofolate adducts, similar experiments were not performed by using protein samples or cellular extracts (i.e., in the presence of various amino acids). In addition, standard curves for predicting formaldehyde concentration in tissues were generated in aqueous solutions rather than biological samples.
is rapidly metabolized or interacts with macromolecules when it escapes metabolism, or is otherwise undetected.

Results from an earlier experiment using radiolabeled formaldehyde in rats are consistent with the conclusion based on the GC-MS measurements of no appreciable increase in blood levels of formaldehyde. Following a 6-hour exposure of F344 rats to 15 ppm of [14C]-formaldehyde (Heck et al., 1983), the concentrations of 14C in the nasal mucosa were 28-fold higher than those in the blood. The observed half-life of the terminal phase of the radioactivity was long (55 hours); on the other hand, it is known that the half-life of free formaldehyde in the rat blood is very short. Therefore, the authors concluded that the radioactivity was likely due to modification of macromolecules or metabolic incorporation rather than slow metabolic clearance of formaldehyde. The terminal decline of the radioactivity in the packed cell fraction of the blood was much slower and observed to be consistent with incorporation into erythrocytes.

In the same paper, Heck et al. (1983) report on the similarity in the pharmacokinetics of radiolabeled formaldehyde and radiolabeled formate in the rat blood, supporting their hypothesis that oxidation of formaldehyde to formate and subsequent incorporation of this compound through one-carbon metabolism were major factors in the disposition of formaldehyde. Studies by Gottschling et al. (1984) have also established that the main product of metabolic clearance of formaldehyde is formate, which is either further metabolized to CO2 and water, incorporated into the one-carbon pool, and/or eliminated in the urine as a sodium salt at about 13 mg/L urine.

3.3.4. Levels in Various Tissues

The radiolabeling studies indicated high levels of 14C in the rat nasal mucosa (equivalent concentrations of 14C-formaldehyde in the nasal mucosa of rats naïvely exposed to 15 ppm 14C-formaldehyde were 2,148 ± 255 nmol/g compared with 76 ± 11 nmol/g in plasma). In contrast, the GC-MS studies did not detect elevated formaldehyde in this region. This is not to be interpreted as a discrepancy, because the radiolabeling study did not distinguish among radiolabeled species and thus the measured radioactivity could potentially be free or bound formaldehyde, formate, or any [14C] metabolically incorporated into macromolecules.

In concurrent studies, Casanova-Schmitz et al. (1984) resolved the question as to whether the higher [14C] levels in the nasal mucosa were a consequence of GSH depletion and a subsequent reduction in GSH-dependent clearance of formaldehyde. An important result in these studies was that there was no significant difference in labeling in either the nasal mucosa or in plasma between naïve F344 rats and those pre-exposed to unlabeled 15 ppm formaldehyde 6 hours/day for the 9 previous days. These findings indicated little or no apparent effect on the
disposition of formaldehyde following short-term exposure to relatively high levels of formaldehyde. In contrast, Farooqui et al. (1986) reported decreases in GSH in several tissues 3 hours after a sublethal I.P. injection of formaldehyde but not after 6 and 9 hours. Taken together, these data suggest that formaldehyde exposure does not result in long-term alterations in cellular GSH levels and that repeated inhalation exposure does not alter the dosimetry to the bloodstream or formaldehyde body burden.

Heck et al. (1983) determined the $^{14}$C concentrations in different tissues in the F344 rat body by exposing rats in a head-only chamber to various concentrations (5–24 ppm) of radiolabeled formaldehyde for 6 hours. (Concentrations of $^{14}$C in internal organs and tissues relative to that in plasma did not appear to vary much as exposure concentrations increased; therefore only averages over the concentration range were reported.) Except for the esophagus, levels in the heart, spleen, lung, intestines, liver, and kidney were 1–3 times higher relative to that in plasma. Labeling in the esophagus was high (fivefold relative to plasma). The authors attributed this relatively higher dose to mucociliary action in the nose and trachea. The data also indicate that the brain, testes, and erythrocytes appear to have about threefold lower $^{14}$C levels than plasma. Pre-exposure to formaldehyde (for 9 days) did not alter the measured radioactivity in the nasal mucosa or plasma. Thus, it was concluded that the single exposure findings may also be qualitatively extended to chronic exposures.

The total radiolabel measured in the bone marrow (femur) of F344-rats exposed for 6 hours to 0.3–15 ppm of radiolabeled formaldehyde in the Casanova et al. (1984) experiment was high (generally within a factor of 0.5 of the total labeling in the nasal respiratory mucosa). Nearly half of the $^{14}$C was contained in the DNA in this tissue presumably on account of the high rate of cell turnover in the bone marrow, indicating that the carbon derived from $^{14}$C-formaldehyde was utilized for DNA synthesis (Casanova-Schmitz et al., 1984).

Chang et al. (1983) described visceral labeling (via autoradiography) in rats, following exposure to 15 ppm [$^{14}$C]-formaldehyde 6 hours/day for 4 days. The authors attributed this labeling to mucociliary clearance and grooming-related ingestion of formaldehyde.

In summary, following exposure to radiolabeled formaldehyde, the radioactivity was very high in the nasal mucosa but was also extensively distributed to various tissues. In particular, levels in the bone marrow were high. On the other hand, formaldehyde levels in the blood measured by GC-MS were not significantly elevated. Thus, the authors considered it unlikely that the elevated $^{14}$C in various tissues was due to free formaldehyde. Instead, these levels were thought to arise from either rapid metabolic incorporation or formation of covalent adducts or incorporation via carboxylation reactions of the $^{14}$CO$_2$ formed during metabolism.
The data presented thus far in this section illustrate that measuring the distribution of the absorbed formaldehyde based on $^{14}$C-radiolabeling and GC-MS studies alone is problematic because it is difficult to resolve (through these studies) whether it is free, reversibly bound, irreversibly bound, formate, one-carbon pool, etc. This is of significance with regard to understanding the availability of the absorbed formaldehyde. More indirect methods had to be developed to further examine the disposition of formaldehyde; however, as discussed below, the interpretation of these approaches may also not be straightforward.

3.3.4.1. Disposition of Formaldehyde: Differentiating Covalent Binding and Metabolic Incorporation

The motivation in presenting this section is twofold, as follows:

1. As concluded above, subsequent studies were necessary to ascertain whether measured radiolabeling in different experiments was due to formaldehyde adducts or incorporation of $[^{14}$C] one-carbon units of formaldehyde into macromolecules via the one-carbon pool.

2. DNA protein cross-links (DPXs) formed by formaldehyde (covalently bound in this case) have been regarded as a surrogate dose metric for the intracellular concentration of formaldehyde (Hernandez et al., 1994; Casanova et al., 1991, 1989). This is particularly relevant because of the nonlinear dose response for DPX formation due to saturation of enzymatic defenses at high concentrations (Casanova et al., 1991, 1989). Thus, the ability to measure DPX is an important development.

An important question is whether the formaldehyde disposed in the form of DPX is detected in remote tissues. A set of elegant but complex experiments involving dual isotope labeling ($^{14}$C and $^{3}$H) was carried out to this end by the Heck and Casanova-Schmitz and their coworkers. Casanova-Schmitz et al. (1984) and Casanova-Schmitz and Heck (1983) used dual isotope labeling of formaldehyde as a way to partially distinguish between formaldehyde adducts formation and metabolic incorporation. In separate experiments, F344 rats were exposed to $^{3}$H- and $^{14}$C-formaldehyde at different exposure concentrations (0.3–15.0 ppm), and the $^{3}$H/$^{14}$C ratios of different phases of DNA were measured. Only the highlights of the results and significant issues are presented here. The overall conclusions from these experiments were as follows:

- Labeling in the nasal mucosa was due to both covalent binding and metabolic incorporation.
- DPX was formed at 2 ppm and greater concentrations in the respiratory mucosa.
- In the bone marrow, formaldehyde did not bind covalently to bone marrow macromolecules at any exposure concentration. The labeling of bone marrow...
macromolecules was found to be entirely due to metabolic incorporation and not due to covalent binding.

Macromolecules such as DNA and protein can be isolated from tissue homogenates by extraction into three phases: an organic phase consisting of proteins, an aqueous phase consisting of only double-stranded DNA, and an interfacial phase consisting of both DNA and protein. Single-stranded (but not double-stranded) DNA was particularly likely to form adducts. DNA from this interfacial phase can be further purified and has been shown to consist of DPXs (Casanova-Schmitz and Heck, 1983). Because both $[^{14}\text{C}]$-formaldehyde and $[^{3}\text{H}]$-formaldehyde can become incorporated into DNA and protein metabolically as well as by cross-linking, the $^{3}\text{H}/^{14}\text{C}$ ratio in such cross-linked material should be higher than in material that primarily contains metabolically incorporated formaldehyde. Figure 3-2 shows the labeling of tissue from the nasal respiratory mucosa and bone marrow (distal femur) in rats exposed to $[^{14}\text{C}]$-formaldehyde and $[^{3}\text{H}]$-formaldehyde vapor.

In the nasal mucosa the interfacial phase has a significantly higher $^{3}\text{H}/^{14}\text{C}$ ratio than the material in the aqueous phase. This suggests that interfacial DNA has significantly more $^{3}\text{H}$, a phenomenon likely explained by additional $[^{3}\text{H}]$-formaldehyde molecules present as DPXs prior to extraction. The amount of interfacial DNA was found to have a clear dose response. These cross-links were also judged to be due to exogenous formaldehyde. Likewise, the organic phase of the nasal mucosa showed a similar increase in $^{3}\text{H}/^{14}\text{C}$ ratio at higher concentrations, a result that could be attributed to various inter- and intraprotein adducts (Metz et al., 2004; Trezl et al., 2003; Skrzydlewska, 1996).

In contrast, analysis of macromolecules at the distal femur location presents a different pattern (see Figure 3-2, part B). First, the interfacial phase was not detected during extraction, suggesting that there were few or no DPXs to be detected. Second, there was no increase in $^{3}\text{H}/^{14}\text{C}$ ratio in the organic (i.e., protein) phase as a function of dose. Therefore, it was concluded that either radiolabeled formaldehyde or formate reached the distal site and was subsequently incorporated into macromolecules. According to the mechanistic interpretation of these studies, the quantity plotted on the ordinate in Figure 3-2 (the ratio of $^{3}\text{H}/^{14}\text{C}$ between the tissue and the exposure gas) should approach unity as metabolism becomes saturated and more adduct formation occurs, particularly for protein. Indeed, this is what is observed (see Figure 3-2, Part A). In contrast, there is no dose effect in the femur, suggesting that the labeling at all doses in that tissue may be due to metabolic incorporation and not due to the parent formaldehyde.
Figure 3-2. $^{3}$H/$^{14}$C ratios in macromolecular extracts from rat tissues following exposure to $^{14}$C- and $^{3}$H-labeled formaldehyde (0.3, 2, 6, 10, 15 ppm).

Note that the small yield of interfacial (IF) phase from bone marrow tissue precluded further analysis; this is prima facie evidence for the lack of significant DPXs in this tissue.

Source: Casanova-Schmitz et al. (1984a).

(Note: These data were originally shown in the absence of an analysis of isotope effects on covalent binding and metabolism. Subsequent studies determined that $[^{3}\text{H}]-\text{formaldehyde}$ is oxidized less rapidly than $[^{14}\text{C}]-\text{formaldehyde}$ and unlabeled formaldehyde. This suggests that the $^{3}\text{H}/^{14}\text{C}$ ratio, and therefore the amount of formaldehyde covalently bound to tissue, is likely overestimated because more $[^{3}\text{H}]-\text{formaldehyde}$ remains unmetabolized, i.e., free to bind [Heck and Casanova, 1987]. The authors hypothesized that this overestimate was relatively greater at the lower concentrations.)

Similar results were obtained in GSH-depleted rats (Casanova and Heck, 1987). Again, these authors observed a dose-dependent increase in the $^{3}\text{H}/^{14}\text{C}$ ratio in the interfacial DNA and organic fractions of disrupted cells of the respiratory and olfactory mucosa and no such increases in bone marrow. Interestingly, at 10 ppm exposure (only), GSH-depleted rats exhibited a higher $^{3}\text{H}/^{14}\text{C}$ ratio in the organic phase than did normal rats. Casanova and Heck (1987) posited that much of the covalent binding at 6 ppm and lower was due to binding to extracellular proteins, whereas the higher $^{3}\text{H}/^{14}\text{C}$ ratio in GSH-depleted rats at 10 ppm was due to more intracellular binding.
In their first experiment to measure DPX concentrations, Casanova-Schmidt et al. (1984) and Casanova and Heck (1987) used the dual isotope method (\(^{3}\text{H}/^{14}\text{C}\)) mentioned above. In this experiment, DPX was observed only at formaldehyde concentrations \(\geq 2\) ppm. Subsequently, Casanova et al. (1989) developed a more sensitive method using high-performance liquid chromatography (HPLC) for measuring DPX. In this method, tissue homogenates were digested with a proteolytic enzyme and extracted with a phenolic solvent. DPX was detected in the nasal mucosa of rats at formaldehyde concentrations as low as 0.3 ppm. This method was also used to measure DPX in the nasal region, the larynx, trachea and carina, and major intrapulmonary airways (airway diameters >2 mm) of rhesus monkeys exposed for 6 hours to 0.7, 2.0, and 6.0 ppm of formaldehyde. DPX was detected in the nose (including the nasopharynx) at all concentrations and at 2.0 and 6.0 ppm in the larynx, trachea, carina, and other lower airways. However, DPX was not detectable in the bone marrow of these monkeys at any concentration.

Overall, Heck and Casanova-Schmitz and their coworkers interpreted the results of these various experiments to mean that inhaled formaldehyde could not reach distant sites in the body. It may be noted in this context that Shaham et al. [1996] reported elevated DPX levels in the white blood cells of laboratory workers exposed to formaldehyde. These data are further reported in Chapter 4.

**3.4. METABOLISM**

Formaldehyde is primarily metabolized by glutathione-dependent formaldehyde dehydrogenase (FALDH) and aldehyde dehydrogenases (ALDHs). Numerous studies now recognize FALDH as a member of the alcohol dehydrogenase (ADH) family, specifically ADH3 (Thompson et al., 2009; Liu et al., 2004, 2001; Hedberg et al., 2003; Høøg et al., 2003; and the references in each of these). The remainder of this report will refer to FALDH as ADH3.

**3.4.1. In Vitro and In Vivo Characterization of Formaldehyde Metabolism**

Formaldehyde is oxidized to formate by two metabolic pathways (see Figure 3-3). The first pathway involves conversion of free formaldehyde to formate by the so-called low-\(K_m\) \((K_m = 400\ \mu\text{M})\) mitochondrial aldehyde dehydrogenase-2 (ALDH2). The second pathway involves a two-enzyme system that converts glutathione-conjugated formaldehyde \((\text{S-hydroxymethylglutathione [HMGSH]})\) to the intermediate S-formylglutathione, which is subsequently metabolized to formate and GSH by S-formylglutathione hydrolase.
Figure 3-3. Formaldehyde clearance by ALDH2 (GSH-independent) and ADH3 (GSH-dependent).

The $K_m$ value for ALDH2 and free formaldehyde is about 400 µM (Teng et al., 2001), whereas the $K_m$ value for HMGSH and ADH3 is 6.5 µM (Uotila and Koivusalo, 1974a, b). The ADH-mediated reactions are reversible in the presence of excess reduced nicotinamide adenine dinucleotide (NADH).

Source: Adapted from Teng et al. (2001).

Though ADH3 is rate limiting in this second pathway, the affinity of HMGSH for ADH3 ($K_m = 6.5$ µM) is about 100-fold higher than that of free formaldehyde for ALDH2. In addition to the kinetic properties, this member of the ADH gene family (Høøg et al., 2003, 2001; Liu et al., 2001; Jornvall et al., 2000; Estonius et al., 1996) appears to be ubiquitously expressed in organ tissues (Molotkov et al., 2002; Ang et al., 1996a, b), exhibits cytoplasmic and nuclear localization (Fernandez et al., 2003), and is the most abundant ADH family member in the liver and brain (Galter et al., 2003).

In vitro studies have examined the clearance of formaldehyde in several human and rat tissues (see Table 3-1). Examination of formaldehyde metabolism in the rat nasal and olfactory mucosa indicates nearly identical pharmacokinetics in the rat liver on a per mg of cell lysate basis (Casanova-Schmitz et al., 1984b). Similar results have been obtained in the absence of GSH, where other ALDH family members oxidize formaldehyde, albeit with significantly lower affinity (i.e., higher $K_m$). Hedberg et al. (2000) demonstrated that human buccal tissue lysate kinetics are in close agreement with those reported for purified human liver ADH3 (Uotila and Koivusalo, 1974a). Additionally, micro-array analysis indicates that these cells express far more ADH3 and S-formylglutathione hydrolase than ALDH1 or ALDH2 (Hedberg et al., 2001a).
results of Ovrebo et al. (2002) are not easily compared with the other studies in Table 3-1 because these studies were in intact cell cultures. However, it is apparent that the pharmacokinetic values in these human cells are comparable to intact rat liver cells.

### Table 3-1. Formaldehyde kinetics in human and rat tissue samples

<table>
<thead>
<tr>
<th>Source</th>
<th>( K_m ) (µM)</th>
<th>( V_{\text{max}} ) (nmol/mg protein × min)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified human liver ADH3</td>
<td>6.5</td>
<td>2.77 ± 0.12</td>
<td>Uotila and Koivusalo (1974a, b)</td>
</tr>
<tr>
<td>Rat olfactory mucosa (+ GSH)</td>
<td>2.6 ± 0.5</td>
<td>1.77 ± 0.12</td>
<td>Casanova-Schmitz et al. (1984b)</td>
</tr>
<tr>
<td>Rat olfactory mucosa (− GSH)</td>
<td>647 ± 43</td>
<td>4.39 ± 0.14</td>
<td>Casanova-Schmitz et al. (1984b)</td>
</tr>
<tr>
<td>Rat respiratory mucosa (+ GSH)</td>
<td>2.6 ± 2.6</td>
<td>0.90 ± 0.24</td>
<td>Casanova-Schmitz et al. (1984b)</td>
</tr>
<tr>
<td>Rat respiratory mucosa (− GSH)</td>
<td>481 ± 88</td>
<td>4.07 ± 0.35</td>
<td>Casanova-Schmitz et al. (1984b)</td>
</tr>
<tr>
<td>Rat liver (+ GSH)</td>
<td>5.0 ± 1.9</td>
<td>2.0 ± 0.3</td>
<td>Casanova-Schmitz et al. (1984b)</td>
</tr>
<tr>
<td>Human bronchial explants(^a)</td>
<td>5,100</td>
<td>3.3</td>
<td>Ovrebo et al. (2002)</td>
</tr>
<tr>
<td>Human bronchial epithelial(^a)</td>
<td>1,400</td>
<td>6.1</td>
<td>Ovrebo et al. (2002)</td>
</tr>
<tr>
<td>Rat hepatocytes(^a)</td>
<td>1,250</td>
<td>4.2</td>
<td>Ovrebo et al. (2002)</td>
</tr>
<tr>
<td>Human buccal tissue (+ GSH)</td>
<td>11 ± 2</td>
<td>2.9 ± 0.6</td>
<td>Hedberg et al. (2000)</td>
</tr>
<tr>
<td>Human buccal tissue (− GSH)</td>
<td>360 ± 90</td>
<td>1.2 ± 0.7</td>
<td>Hedberg et al. (2000)</td>
</tr>
<tr>
<td>Human keratinocytes</td>
<td>n.d.(^b)</td>
<td>14.5 ± 1.8</td>
<td>Hedberg et al. (2000)</td>
</tr>
<tr>
<td>Human fibroblasts</td>
<td>n.d.</td>
<td>17.9 ± 1.4</td>
<td>Hedberg et al. (2000)</td>
</tr>
</tbody>
</table>

\(^a\)These studies were carried out in intact cells by measuring the formation of formate. This likely explains the nearly 1,000-fold increase in apparent \( K_m \), since much of the formaldehyde was likely to be bound extracellularly. The remaining studies used either purified enzyme or cell lysates (as indicated) and measured the formation of NADH.

\(^b\)n.d. = not determined.

The data in Table 3-2 along with data indicating the ubiquity of ADH3, indicate that many human tissues and cells, particularly in the respiratory tract, appear to exhibit significant capacity to metabolize formaldehyde. Molecular biology techniques have demonstrated the importance of ADH3 in formaldehyde clearance. For example, ADH-knockout studies have shown that the median lethal dose (LD\(_{50}\)) values for formaldehyde in wild type, ADH1\(^{-/-}\), ADH3\(^{-/-}\), and ADH4\(^{-/-}\) mice strains were 0.200, 0.175, 0.135, and 0.190 g/kg, respectively (Deltour et al., 1999). Although the statistical significance was not reported, the data indicate that deletion of ADH3 increases the sensitivity of mice to formaldehyde.
Table 3-2. Allelic frequencies of ADH3 in human populations

<table>
<thead>
<tr>
<th>Population, n</th>
<th>Allele frequencies (%)</th>
<th>AA&lt;sub&gt;197-196&lt;/sub&gt;</th>
<th>GG&lt;sub&gt;197-196&lt;/sub&gt;</th>
<th>A&lt;sub&gt;79&lt;/sub&gt;</th>
<th>G&lt;sub&gt;79&lt;/sub&gt;</th>
<th>T&lt;sub&gt;69&lt;/sub&gt;</th>
<th>C&lt;sub&gt;69&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese, 83</td>
<td></td>
<td>22</td>
<td>78</td>
<td>100</td>
<td>–</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>Spanish, 95</td>
<td></td>
<td>41</td>
<td>59</td>
<td>62</td>
<td>38</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>Swedish, 96</td>
<td></td>
<td>47</td>
<td>53</td>
<td>67</td>
<td>33</td>
<td>1.5</td>
<td>98.5</td>
</tr>
</tbody>
</table>

Source: Adapted from Hedberg et al. (2001b).

The pharmacokinetics of formate are complex. Formate can undergo adenosine triphosphate (ATP)-dependent addition to tetrahydrofolate (THF), which can carry either one or two one-carbon groups. Formate can conjugate with THF to form N<sup>10</sup>-formyl-THF and its isomer N<sup>5</sup>-formyl-THF, both of which can be converted to N<sup>5</sup>,N<sup>10</sup>-methenyl-THF and subsequently to other derivatives that are ultimately incorporated into DNA and proteins via biosynthetic pathways (see Figure 3-4).

Figure 3-4. Metabolism of formate.

Note: 1, formyl-THF synthetase; 2, formyl-THF dehydrogenase.

Source: Adapted from Black et al. (1985).
Elevated levels of formate in urine have been detected following inhalation of methanol or formate under certain conditions (Liesivuori and Savolainen, 1987), although the interpretation of this finding is unclear. There is also evidence that formate generates CO₂⁻ radicals and can be metabolized to CO₂ via catalase and via the oxidation of N¹⁰-formyl-THF (Dikalova et al., 2001, and references therein). The significance of formate in formaldehyde toxicity is unclear. Black et al. (1985) reported that hepatic tetrahydrofolate levels in monkeys are 60% of those in rats and that primates are far less efficient in clearing formate than are rats and dogs. Studies in rats involving [¹⁴C]-formate suggest that about 80% is exhaled as ¹⁴CO₂, 2–7% is excreted in the urine, and about 10% undergoes metabolic incorporation (Hanzlik et al., 2005, and references therein). Mice deficient in formyl-THF dehydrogenase exhibit no change in LD₅₀ (via I.P. dose) for methanol or in oxidation of high doses of formate (Cook et al., 2001). It has been suggested that rodents efficiently clear formate via folate-dependent pathways, peroxidation by catalase, or an unknown third pathway. Conversely, primates do not appear to exhibit such capacity and are more sensitive to metabolic acidosis following methanol poisoning (Cook et al., 2001).

3.4.2. Formaldehyde Exposure and Perturbation of Metabolic Pathways

The enzyme ADH3 has received renewed attention in recent years because of new functions that have been attributed to it. ADH3 is central to the metabolism of formaldehyde; however, exposure to formaldehyde in turn alters the activity of ADH3 (in multiple dose-dependent ways), thereby leading to perturbation of critical metabolic pathways. These are briefly mentioned below (refer to cited papers for details).

1. Exposure to formaldehyde increases cell replication. These proliferating epithelial and inflammatory cells are rich in both the messenger ribonucleic acid (mRNA) and protein of ADH3 (Nilsson et al., 2004; Hedberg et al., 2000). Studies in the rodent lung suggest that increases in ADH3 in such cells dramatically alter the biology of other important ADH3 substrates that are involved in protein modification and cell signaling (Que et al., 2005).

2. ADH3 also participates in the oxidation of retinol and long-chain primary alcohols, as well as the reduction of S-nitrosoglutathione (GSNO) (Staab et al., 2009; Thompson et al., 2009; Hedberg et al., 2003; Hoøg et al., 2003; Molotkov et al., 2002; Liu et al., 2001; Jornvall et al., 2000; Jensen et al., 1998). The activity of ADH3 toward some of these substrates has been shown to be significantly increased in the presence of formaldehyde. Staab et al. (2009) showed that (in cultured cells) GSNO can accelerate ADH3-mediated formaldehyde oxidation and, likewise, that formaldehyde increases ADH3-mediated GSNO reduction nearly 25-fold. The following effects may be noted with regard to the relevance of such perturbations.

This document is a draft for review purposes only and does not constitute Agency policy.
a. GSNO is an endogenous bronchodilator and reservoir of nitric oxide (NO) activity (Jensen et al., 1998). Details on the ADH3-mediated reduction of GSNO are shown in Thompson and Grafstrom (2008).

b. ADH3 is implicated in playing a central role in regulating bronchiole tone and allergen-induced hyperresponsiveness (Gerard, 2005; Que et al., 2005).

c. As concluded by California Environmental Protection Agency (CalEPA) (2008), “the dysregulation of NO by formaldehyde [in this manner] helps to explain the variety and variability in the toxic manifestations following formaldehyde inhalation.”

3.4.3. Evidence for Susceptibility in Formaldehyde Metabolism

Teng et al. (2001) provided evidence that inhibition of ADH1, ALDH2, and ADH3 has significant impact on formaldehyde toxicity. The authors speculated that deficiencies in any of these enzymes would confer an increased susceptibility to formaldehyde toxicity (Teng et al., 2001). Polymorphism in ALDH2 has been shown to have implications in human risk assessment, specifically with regard to acetaldehyde metabolism (Ginsberg et al., 2002). It is worth noting, however, that Teng et al. (2001) only demonstrated the importance of ALDH2 in rat hepatocytes with formaldehyde concentrations of 2.5 mM and greater. Since this concentration is fivefold greater than the 0.5 mM $K_m$ for free formaldehyde, ALDH2 involvement is not unexpected at such high concentrations. Teng et al. (2001) also demonstrated the importance of ADH1 in driving the reverse reaction (i.e., formaldehyde to methanol) by coadministration of NADH-generators. This would have the effect of prolonging the life of formaldehyde by continuous recycling. This is not surprising, given that many ADH reactions are reversible. However, levels of nicotinamide adenine dinucleotide (NAD$^+$) are normally much higher than NADH.

To date, two studies have reported polymorphisms in ADH3, using the new nomenclature. ADH3 transcription appears to be regulated by specificity protein (Sp1), with a minimal promoter located at positions $-34$ to $+61$. The reported polymorphisms in ADH3 involve four base-pair substitutions in the promoter region and no polymorphisms in the coding region (Hedberg et al., 2001b). The three polymorphisms include $-197/-196$ (GG$\rightarrow$AA), $-79$ (G$\rightarrow$A), and $+9$ (C$\rightarrow$T). The genotype frequencies are shown in Table 3-2. Of these alleles, the $+9$ (C$\rightarrow$T) polymorphism (in the putative Sp1 minimal promoter region) reduced transcriptional

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5 Other epidemiologic studies investigating links between ADH3 and oral cancer use the older nomenclature and thus refer to Class I ADH (i.e., ADH1) enzymes.
activity twofold in in vitro reporter gene experiments. According to Hedberg et al. (2001b), no
studies have demonstrated differences in ADH3 enzyme activity in humans. More recently,
single nucleotide polymorphisms in ADH3 have been reported to be associated with childhood
risk of asthma, although the functional relevance of these polymorphisms has not been published
(Wu et al., 2007).

Alterations in THF pathways may also have an impact on formaldehyde toxicity. These
could result from polymorphisms in various enzymes or differences in folate intake and
absorption. Species differences in tetrahydrofolate levels (Black et al., 1985) are thought to play
a role in the differential responses to methanol across species. Cook et al. (2001) speculate that
rats have redundant pathways for formate clearance that may be absent or less efficient in
primates.

3.5. ENDOGENOUS SOURCES OF FORMALDEHYDE

Endogenous formaldehyde is produced through normal cellular metabolism through
enzymatic or nonenzymatic reactions, and also as a detoxification product of xenobiotics during
cellular metabolism.

3.5.1.1. Normal Cellular Metabolism (Enzymatic)

Formaldehyde is produced during normal metabolism of methanol, amino acids (e.g.,
glycine, serine, and methionine), choline, dimethylglycine, and methylamine and through the
folate-dependent endogenous one-carbon pool, etc.

a) One of the endogenous sources for formaldehyde production is methanol, formed during
normal cellular metabolism. However, this fraction may also be derived through
consumption of fruits, vegetables and alcohol (Shelby et al., 2004; IPCS, 1997). In
studies conducted with healthy humans whose diet was devoid of methanol-containing or
methanol-generating foods (such as cereals containing aspartame, a precursor of
methanol) and who abstained from alcohol consumption, the background blood levels of
methanol range from 0.25–4.7 mg/L (reviewed in Shelby et al., 2004 [CERHR]).
Methanol is metabolized to formaldehyde predominantly by hepatic alcohol
dehydrogenase-1 (ADH1) in primates and by ADH1 and catalase (CAT) in rodents,
ADH1 requiring nicotinamide adenine dinucleotide (NAD+) as a cofactor.

b) Dimethylglycine (DMG), one of the byproducts of choline metabolism endogenously
present in the body, is an indirect source of endogenous formaldehyde. Two specific
dehydrogenases, (a) dimethylglycine dehydrogenase (DMGDH) which converts DMG to
sarcosine (methylglycine) and (b) sarcosine dehydrogenase (SDH) which converts
sarcosine to glycine, have been shown to noncovalently bind to the folate enzyme,
tetrahydrofolate (THF). Further, these dehydrogenases form “active formaldehyde” by removing the 1-carbon groups from THF (Binzak et al., 2000).

c) Another source of endogenous formaldehyde is methylamine (MA), an intermediary component of the metabolism of adrenaline, sarcosine, creatine, lecithin, and other dietary sources (Yu and Zuo, 1996). The enzyme semicarbozole-sensitive amine oxidase (SSAO), predominantly present in the plasma membrane of endothelial smooth muscle cells and in circulating blood, converts methylamine to formaldehyde, hydrogen peroxide and ammonia. The formaldehyde thus released has been shown to cause endothelial injury eventually leading to atherosclerosis (Kalasz, 2003). Yu et al. (1997) have shown that adrenaline, released in the body as a response to stress, is known to be deaminated by the enzyme monoamine oxidase, with further conversion of methylamine to formaldehyde by SSAO (Yu et al., 1997). Creatine is another precursor for methylamine which is metabolized by SSAO to form formaldehyde. It has been shown that short-term, high-dose dietary supplementation of creatine in healthy humans causes a significant increase in urinary methylamine and formaldehyde levels (Poortmans et al., 2005).

d) Endogenous formaldehyde is also a constituent of the one-carbon pool, a network of interrelated biochemical reactions that involve the transfer of one-carbon groups from one compound to another (usually the transfer of the hydroxymethyl group of serine to tetrahydrofolic acid).

Tyihak et al. (1998) have demonstrated that formaldehyde, but not the methyl radical or methyl cation, is involved in the enzymatic transmethylation and demethylation reactions, and suggested the presence of a formaldehyde cycle in cells for the production and removal of formaldehyde utilizing the transfer through methionine → S-adenosylmethionine → S-adenosyl-homocysteine → homocysteine (Tyihak et al., 1998). However, these studies did not clearly show whether the formaldehyde released in this cycle is in free or bound form.

Formaldehyde has been shown to be produced in normal and leukemic leukocytes from N⁵-methyl-THF by enzymatic degradation (Thorndike and Beck, 1977). This is a two-step reaction involving (1) enzymatic conversion of the methyl-THF to formaldehyde followed by (2) nonenzymatic reaction of formaldehyde with an amine. Thorndike and Beck (1977) showed that leukocyte (granulocyte and lymphocyte) cell extracts from normal individuals and patients with chronic lymphocytic leukemia (CLL) or chronic myelocytic leukemia (CML) incubated with ¹⁴C-methyl-THF and saturating amounts of tryptamine produced free formaldehyde which is detected as its corresponding carboline derivative formed with tryptamine. These results demonstrate the activity of the enzyme N⁵, N¹⁰-methylene THF reductase which oxidizes N⁵-methyltetrahydrofolate to N⁵, N¹⁰ methylene THF. The authors noted that the enzyme levels were in the order of normal granulocytes < normal lymphocytes < granulocytes from a CML individual < lymphocytes from a CLL individual (Thorndike and Beck, 1977), suggesting
increased activity of formaldehyde producing enzyme in leukemic cells compared to normal leukocytes. Overall, formaldehyde might be a byproduct as well as an intermediary product in several of these reactions.

### 3.5.1.2. Normal Metabolism (Nonenzymatic)

1. Formaldehyde can also be formed nonenzymatically by the spontaneous reaction of methanol with hydroxyl radicals, wherein cellular hydrogen peroxide is the precursor for hydroxyl radicals generated through Fenton reaction (Cederbaum and Qureshi, 1982).

2. Another mechanism of nonenzymatic production of formaldehyde is through lipid peroxidation of polyunsaturated fatty acids (PUFA) (Shibamoto, 2006; Slater, 1984). In this mechanism, reactive oxygen species (ROS) generated during oxidative stress abstract a hydrogen atom from a methylene group of polyunsaturated fatty acids (PUFA) in cell membranes causing autooxidation of lipids with the eventual production of free radicals (e.g., peroxy radical). It is known that a certain level of oxidative stress and lipid peroxidation does occur in normal individuals, and these cellular metabolic processes are likely to contribute to endogenous formaldehyde production.

### 3.5.1.3. Exogenous Sources of Formaldehyde Production

Microsomal cytochrome P450 enzymes catalyze oxidative demethylation of N-, O- and S-methyl groups of xenobiotic compounds whereby formaldehyde is produced as a primary product, which is subsequently incorporated into the one-carbon pool by reacting with tetrahydrofolate acid or is oxidized to formate (Dahl and Hadley, 1983; Heck et al., 1982). Also, some special peroxidases, such as peroxide-dependent horseradish peroxidase enzymatically catalyze xenobiotics to generate formaldehyde in the body. In particular, an ethyl peroxide-dependent horseradish peroxidase has been shown to act on N,N-dimethylaniline and produce equimolar amounts of N-methylaniline and formaldehyde (Kedderis and Hollenberg, 1983).

The tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), is another source of formaldehyde. It has been shown that formaldehyde is also produced during the methyl hydroxylation of NNK by rat liver microsomes (Castonguay et al., 1991). Also recent studies have demonstrated the formation of formaldehyde-DNA adducts in NNK-treated rats using a highly sensitive liquid chromatography-electrospray ionization-tandem mass spectrometry with selected reaction monitoring (Wang et al., 2007), suggesting formation of formaldehyde from nitrosamines. Cigarette smoke is also a source of exogenously produced methylamine which is converted to formaldehyde by SSAO (Yu, 1998).
3.5.1.4. Metabolic Products of Formaldehyde Metabolism (e.g., Formic Acid)

Formate is converted to carbon dioxide (CO₂) in rodents predominantly by a folate-dependent enzyme pathway (Dikalova et al., 2001). Formate is also oxidized to CO₂ and water by a minor pathway involving catalase located in rat liver peroxisomes (Waydhas et al., 1978; Oshino et al., 1973). In the folate-dependent pathway, tetrahydrofolate (THF)-mediated oxidation of formate and the transfer of one-carbon compounds between different derivatives of THF has been described.

Endogenous levels of formate also will be affected by dietary intake of methanol-producing or methanol-containing diets since methanol is initially converted to formaldehyde and eventually metabolized to formate. It has been shown in several studies in human subjects who were restricted on consuming methanol producing diets, aspartame or alcohol, that the endogenous blood concentrations of formate ranged from 3.8 to 19.1 mg/L (Shelby et al., 2004 [CERHR]). The biological half life of formic acid is 77–90 minutes (Owen et al., 1990b). The levels of formate in the urine of unexposed individuals range from 11.7 to 18 mg/L (Boeniger, 1987). One source of formic acid intake is through diet which ranges from 0.4 to 1.2 mg per day (Boeniger, 1987). The half life for plasma formate is ~30 minutes or longer (Boeniger, 1987).

3.5.1.5. Levels of Endogenous Formaldehyde in Animal and Human Tissues

Heck et al. (1982) estimated that endogenous levels of formaldehyde (free as well as bound) in rats ranged from 0.05 to 0.5 µmole/g (1.5–15 µg/g) of wet tissue as analyzed by the stable isotope dilution with GC-MS method (Heck et al., 1982). Although the levels of free formaldehyde cannot be measured due to their high reactivity and short half life, they were calculated by Heck et al. (1985) using an indirect method. They added a molar excess of GSH or THF to the test tube containing formaldehyde in aqueous solution enabling complete binding. When estimated, they observed that the amount of formaldehyde detected was equal to the total amount added to the reaction suggesting that the formaldehyde measured contained both free and bound forms. Further, they calculated the free formaldehyde concentration using the dissociation constant of the HMGSH adduct and cellular concentration of GSH. Human formaldehyde dehydrogenase has been shown to have a dissociation constant of 1.5 mM for the formaldehyde-GSH hemithioacetal adduct (Uotila and Koivusalo, 1974), while the folate enzyme product N⁵,N¹⁰-methylene-THF has a dissociation constant of 30 mM (Kallen and Jencks 1966a, b). This could be evaluated using the Michaels-Menton constant (Kₘ) of formaldehyde dehydrogenase for the GSH adduct (~4 µM at 25°C), whereby they calculated the free formaldehyde level to be around 3–7 µM or 1–2% of the total formaldehyde as measured by GC-MS in rat tissues (Heck 1982).
Cascieri and Clary (1992) estimated the total body content of formaldehyde in human body based on the following assumptions. For an individual with an average body wt of 70 kg and with body fluids accounting for 70% of body weight, total formaldehyde content is distributed in ~49 kg of body mass or 49 L of body fluids, owing to the water solubility and uniform distribution of formaldehyde in body fluids. It has been shown that the average blood concentration (mean ± S.E.) of formaldehyde in unexposed rats and humans was 2.24 ± 0.07 and 2.61 ± 0.14 µg/g of blood, respectively (Heck et al., 1985), and in unexposed rhesus monkeys it was 2.42 ± 0.09 µg/g of blood (Casanova et al., 1988), overall giving an average of approximately 2.5 ppm (2.5 mg/L) formaldehyde across the species. All these studies used pentafluorophenyl hydrazine derived formaldehyde using GC-MS analysis (see Table 3-3).

Assuming these values, the body content of total formaldehyde is 122.5 mg (49 L × 2.5 mg/L) or 1.75 mg/kg body wt at any given time. Formaldehyde given intravenously to rhesus monkeys has been shown to have a half life of ~1.5 minutes in blood, wherein formaldehyde in blood was measured by the dimedone method (McMartin et al., 1979). Using this information Cascieri and Clary (1992) calculated that the human body generates approximately 40.83 mg/minute [(122.5 mg/2 × 1.5] of formaldehyde. Biotransformation of formaldehyde to carbon dioxide in the liver alone has been estimated at 22 mg/minute (Owen et al., 1990a).

Free formaldehyde is detected in body fluids and tissues using dimedone (Szarvas et al., 1986) or 2,4-dinitrophenylhydrazine (DNPH) or pentafluorophenyl hydrazine (PFPH) derivative (Heck et al., 1985) or as a fluorescent derivative (Luo et al., 2001) as trapping agent and detected by analytical techniques such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and gas-chromatography mass spectrometry (GC-MS). Data from several studies is summarized in Table 3-3. Using 14C-labeled dimedone, a chemical which condenses with free formaldehyde forming a product termed “formaldemethone” enabling radiometric detection, Szarvas et al. (1986) estimated the levels of endogenous formaldehyde in human blood plasma to be 0.4–0.6 µg/mL and in human urine to be 2.5–4 µg/mL (Szarvas et al., 1986).

Hileman (1984) reported that the endogenous levels of metabolically derived formaldehyde will be in the range of 3–12 ng/g of tissue (Hileman, 1984). So for an average 70 kg individual, the endogenous level of metabolically derived formaldehyde would be 210 µg to 840 µg (3–12 ng/g × 0.001 µg/ng × 1,000 g/kg × 70 kg).

Table 3-3. Endogenous formaldehyde levels in animal and human tissues and body fluids

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Method</th>
<th>Detected as</th>
<th>Formaldehyde levels</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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### Table: Formaldehyde Levels in Various Biological Matrices

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Analytical Method</th>
<th>Adduct Form</th>
<th>Quantitation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not specified</td>
<td>Not specified</td>
<td>Not specified</td>
<td>0.003–0.012 ppm (3–12 ng/g)</td>
<td>Hileman 1984</td>
</tr>
<tr>
<td>Not specified</td>
<td>GC-MS with stable isotope dilution method</td>
<td>As PFPH-derivative</td>
<td>1.5–15 ppm (0.05–0.5 µmole/g)</td>
<td>Heck et al., 1982a</td>
</tr>
<tr>
<td>Blood</td>
<td>GC-MS with select ion monitoring</td>
<td>As PFPH-derivative</td>
<td>2.24 ± 0.07 ppm (2.24 ± 0.07 µg/g)</td>
<td>Heck et al., 1985</td>
</tr>
<tr>
<td>Blood</td>
<td>GC-MS with select ion monitoring</td>
<td>As PFPH-derivative</td>
<td>2.61 ± 0.14 ppm (2.61 ± 0.14 µg/g)</td>
<td>Heck et al., 1985</td>
</tr>
<tr>
<td>Plasma</td>
<td>Reverse phase HPLC-fluorescent detection</td>
<td>As product of ampicillin</td>
<td>1.65 ppm (1.65 µg/mL)</td>
<td>Luo et al., 2001</td>
</tr>
<tr>
<td>Heart perfusate</td>
<td>HPLC</td>
<td>As DNPH adduct</td>
<td>0.089–0.126 ppm (2.98–4.21 nmol/mL)</td>
<td>Shibamoto 2006</td>
</tr>
<tr>
<td>Blood</td>
<td>GC-MS with select ion monitoring</td>
<td>As PFPH-derivative</td>
<td>2.42 ± 0.09 ppm (2.42 ± 0.09 µg/g)</td>
<td>Casanova et al., 1988</td>
</tr>
<tr>
<td>Plasma</td>
<td>Radiometric method</td>
<td>As formaldehyde-methone adduct</td>
<td>0.4–6 ppm (0.4–6 µg/mL)</td>
<td>Szarvas et al., 1986</td>
</tr>
<tr>
<td>Urine</td>
<td>Radiometric method</td>
<td>As formaldehyde-methone adduct</td>
<td>2.5–4.0 ppm (2.5–4.0 µg/mL)</td>
<td>Szarvas et al., 1986</td>
</tr>
</tbody>
</table>

Values in the parenthesis, originally cited in the references, are converted to parts per million (ppm) as indicated. PFPH, pentafluorophenyl hydrazone derivative; DNPH, dinitrophenyl hydrazine; GC-MS, gas-chromatography mass spectrometry; HPLC, high performance liquid chromatography.

### 3.6. EXCRETION

The main product of metabolic clearance of formaldehyde is formate, which is further metabolized to CO₂ and water, incorporated into the one-carbon pool, or eliminated in the urine. There is also some evidence that formaldehyde is present in exhaled breath; however, it is unclear whether this originates from endogenous sources, or is simply a function of ambient formaldehyde dissolved in fluids lining POEs. The following sections describe first experiments in laboratory species and then available data in humans. Broadly, these studies address two important questions that may be of relevance for risk assessment. First, it may be of interest to know what levels of formaldehyde are exhaled for comparison with inhaled levels, and whether there is any relationship between external exposure and exhaled levels. Second, there are recent studies that have attempted to relate genetic polymorphisms and changes in gene transcription level to levels of putative urinary formaldehyde biomarkers.
3.6.1. Formaldehyde Excretion in Rodents

Heck et al. (1983) determined the relative contributions of various excretion pathways in F344 rats following inhalation exposure to formaldehyde. Table 3-4 indicates that the relative excretion pathways were independent of exposure concentration (at least between 0.63 and 15 ppm). Nearly 40% of inhaled $[^{14}\text{C}]$-formaldehyde appeared to be eliminated via expiration, probably as CO$_2$ (it should be recalled that nearly 100% of inhaled formaldehyde is absorbed). Within 70 hours of a 6-hour exposure to formaldehyde, about 17 and 5% were eliminated in the urine and feces, respectively. Nearly 40% of inhaled $[^{14}\text{C}]$-formaldehyde remained in the carcass, presumably due to metabolic incorporation.

Table 3-4. Percent distribution of airborne $[^{14}\text{C}]$-formaldehyde in F344 rats

<table>
<thead>
<tr>
<th>Source</th>
<th>Concentration of formaldehyde (ppm)</th>
<th>Concentration of formaldehyde (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distribution (%)$^a$</td>
<td>Distribution (%)$^a$</td>
</tr>
<tr>
<td>Expired air</td>
<td>39.4 ± 1.5</td>
<td>41.9 ± 0.8</td>
</tr>
<tr>
<td>Urine</td>
<td>17.6 ± 1.2</td>
<td>17.3 ± 0.6</td>
</tr>
<tr>
<td>Feces</td>
<td>4.2 ± 1.5</td>
<td>5.3 ± 1.3</td>
</tr>
<tr>
<td>Tissues and carcass</td>
<td>38.9 ± 1.2</td>
<td>35.2 ± 0.5</td>
</tr>
</tbody>
</table>

$^a$Values are means ± standard deviations ($n = 4$).

Source: Heck et al. (1983).

Mashford and Jones (1982) examined elimination pathways of formaldehyde in rats exposed by I.P. injection. Urine and exhaled gases were collected from rats exposed to 4 or 40 mg/kg $[^{14}\text{C}]$-formaldehyde. At 48 hours postinjection, 82 and 78% of the radiolabel were exhaled as $^{14}\text{CO}_2$, whereas exhaled $[^{14}\text{C}]$-formaldehyde was not detected. Mashford and Jones (1982) also further identified the urinary metabolites. Five hours after injection of the higher dose, formate was determined to comprise 80% of the urinary metabolites. The authors were unable to detect cysteine derivatives observed in other studies (see below) in the urine of these rats prior to or after formaldehyde exposure. The authors stated that if formaldehyde were to be excreted in urine containing cysteine, then thiazolidine-4-carboxylate (TZCA) would likely be produced. They speculated that species differences in urinary compounds may produce formaldehyde conjugates (or artifacts).
Hemminki (1982) reacted formaldehyde and acetaldehyde with cysteine, N-acetylcysteine, and GSH and found that formaldehyde reacted most rapidly with cysteine to form TZCA. Similarly, acetaldehyde reacted preferentially with cysteine, albeit slower than formaldehyde, to form a thiazolidine derivative. However, when each aldehyde was administered I.P. (10% formaldehyde, 50% acetaldehyde), thioether concentrations (nmol/mol creatinine) significantly increased in the 24 and 48 hour urine of acetaldehyde-treated rats but not formaldehyde-treated rats. These data suggest that formaldehyde is not appreciably excreted in urine and thus cysteine conjugates are not likely to represent formaldehyde exposure.

Most recently, Shin et al. (2007) attempted to show that formaldehyde inhalation increased urinary TZCA levels in Sprague-Dawley rats. Treated rats were exposed to 3.1 and 38.1 ppm formaldehyde for 6 hours/day for 2 weeks, and urine was collected for 3 days. The TZCA level in four control rats was 0.07 ± 0.02 mg/L, whereas levels in the 3 and 38 ppm groups were 0.18 ± 0.045 and 1.01 ± 0.36, respectively. Notably, the concentrations in the four highest exposed animals (0.71, 0.70, 1.20, and 1.43 ppm) exhibited a nearly twofold range. However, these comparisons are confounded if the exposures have any influence on urine production and urine cysteine levels. The study does not provide any data that might allow one to examine this issue.

3.6.2. Formaldehyde Excretion in Exhaled Human Breath

Several human and animal studies have attempted to measure the concentration of formaldehyde in exhaled breath. However, study design and limitations of available analytical techniques have resulted in little data which provide a basis for determining levels of formaldehyde in exhaled breath either from normal metabolism (in humans), or when formaldehyde is administered (animal study). The two major limitations of studies of human breath include the potential for false positives for formaldehyde from the primary analytical technique for breath analysis and the need for concurrent room air controls.

A recent study has illustrated that the use of proton transfer reaction in SIFT-MS may result in false positive results for formaldehyde as the characteristic analytical product ion for formaldehyde is also produced from methanol and ethanol found in exhaled breath (Španěl and Smith, 2008). Proton transfer reaction mass spectrometry (PTR-MS) has been applied to measure trace compounds in exhaled breath including volatile organics and specifically formaldehyde. The basic method of PTR-MS is based on the transfer of protons from H$_3$O$^+$ to gases in exhaled breath and the in-line monitoring of products where gases are tentatively identified by the mass to charge ratio ($m/z$) where an $m/z$ of 31 is consistent with protonated formaldehyde (Hansel et al., 1995; Lindinger et al., 1998). It is important to note that reaction
products from methanol and ethanol may also produce fragments with an \( m/z \) ratio of 31 (Kusch et al., 2008). Selected ion flow tube mass spectrometry (SIFT-MS) is an application of PTR-MS developed for real-time analysis of trace gases in breath (Smith and Španěl, 2005; Španěl and Smith, 2007). As shown in Figure 3-5 up to 1% of the mass of ethanol and methanol in exhaled breath may be detected with a mass-to-charge ratio (\( m/z \) ratio) of 31—which may have been reported as formaldehyde in earlier publications (Kusch et al., 2008; Španěl and Smith, 2008). The authors have improved the SIFT-MS software used in exhaled breath analysis to adjust the reported formaldehyde levels by accounting for the contribution of methanol and ethanol to the characteristic analytical product ion for formaldehyde (\( m/z = 31 \)). No published articles were available on formaldehyde in exhaled breath which adjusted for methanol and ethanol levels in exhaled breath. Therefore, the available articles discussed below will be evaluated with respect to the potential for ethanol or methanol to influence the reported formaldehyde levels.

Figure 3-5. Detection of the characteristic analytical product ion for formaldehyde (\( m/z \) ratio of 31) by proton transfer reaction mass spectrometry (PTR-MS) in gas samples spiked with only methanol and ethanol. Open circles show the reported formaldehyde without adjustment for the methanol and ethanol present (each of which produces a small fraction of the analytical product with an \( m/z \) ratio of 31). Closed circles represent the same data, corrected by the SIFT-MS software to control for methanol and ethanol.

Source: Španěl and Smith (2008).
Six articles were located which reported formaldehyde levels in exhaled breath, three of which provide level of methanol and ethanol in exhaled breath in the same individuals or study group and are further discussed below (Wang et al., 2008; Cáp et al., 2008; and Moser et al., 2005). Although Wehinger et al., (2007) report a compound tentatively identified as formaldehyde correlated with a diagnosis of lung cancer, the PTR-MS was not controlled for any contribution of ethanol and methanol, and the levels of these compounds were not provided for comparison so it is not further discussed here. Turner et al. (2008) measured levels of volatile compounds including formaldehyde in exhaled breath five healthy males. The subjects fasted overnight, and measurements were taken before and after ingesting 75 g of glucose. The source of the inhaled air was laboratory air which contained an unreported concentration of formaldehyde. Formaldehyde was not detected in the exhaled breath of any subjects (5 ppb limit of detection) ethanol and methanol levels were not reported.

In a study designed to compare volatile organics in exhaled breath of smokers and nonsmokers, compounds tentatively identified as formaldehyde and methanol were not different between the populations (Kushch et al., 2008). The authors acknowledge that the reported formaldehyde (m/z = 31) might also represent fragments of reaction products from methanol and ethanol. Reported formaldehyde levels were approximately 5% of the methanol (e.g., mean of 9.9 ppb versus 208 ppb respectively).

Wang et al. (2008) measured the concentrations volatile organics, including formaldehyde, in the exhaled breath through the nose or mouth, and oral cavity during breath holding of three healthy male laboratory workers. Measurements were taken in each individual over a period of a month, 20 workdays. Formaldehyde levels (4−7 ppb) were lower than the inspired laboratory air (9.6 ppb) (see Table 3-5). Formaldehyde in the mouth during breath holding, did not differ from the exhaled air (nose or mouth). The SIFT-MS analysis did not adjust for any contribution of ethanol or methanol to the tentatively identified formaldehyde levels. Although only means are reported, a comparison of results in Table 3-5 does indicate that 1% of the reported ethanol and methanol may have contributed significantly to the reported formaldehyde levels.

Cáp et al. (2008) evaluated relationships between volatile organic compounds measured in exhaled breath and exhaled breath condensate. Exhaled breath condensate consists of aerosolized particles of airway lining fluid evolved from the airway wall by turbulent airflow that serve as seeds for substantial water vapor condensation, which then serves to trap water soluble volatile gases. This study also attempted to ascertain whether the source of each compound was endogenous or exogenous. According to the published article and electronic
communication with Dr. Patrik Španěl, a coauthor for this study, the limit of quantification was 3 ppb or better.

Table 3-5. Measurements of exhaled formaldehyde concentrations in the mouth and nose, and in the oral cavity after breath holding in three healthy male laboratory workers. The median levels are estimated as the geometric mean with the associated standard deviation (σ.).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Methanol (median ppb/σ)</th>
<th>Ethanol (median ppb/σ)</th>
<th>Formaldehyde (median ppb/σ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Mouth</td>
<td>178/1.2</td>
<td>236/1.6</td>
<td>5/2.3</td>
</tr>
<tr>
<td>Nose</td>
<td>167/1.2</td>
<td>28/1.3</td>
<td>7/2.1</td>
</tr>
<tr>
<td>Oral cavity</td>
<td>149/1.2</td>
<td>412/1.4</td>
<td>5/2.3</td>
</tr>
<tr>
<td>B Mouth</td>
<td>300/1.4</td>
<td>64/1.6</td>
<td>7/2.3</td>
</tr>
<tr>
<td>Nose</td>
<td>396/1.4</td>
<td>27/1.4</td>
<td>5/2.1</td>
</tr>
<tr>
<td>Oral cavity</td>
<td>358/1.4</td>
<td>93/1.4</td>
<td>6/1.9</td>
</tr>
<tr>
<td>C Mouth</td>
<td>228/1.5</td>
<td>153/1.5</td>
<td>4/2.5</td>
</tr>
<tr>
<td>Nose</td>
<td>229/1.5</td>
<td>26/1.4</td>
<td>6/1.9</td>
</tr>
<tr>
<td>Oral cavity</td>
<td>162/1.7</td>
<td>163/1.4</td>
<td>6/1.9</td>
</tr>
<tr>
<td>Laboratory air</td>
<td>44 ± 9</td>
<td>101 ± 52</td>
<td>9.6 ± 1.5</td>
</tr>
</tbody>
</table>

Notes: The limit of quantification for formaldehyde was not reported.

However, the SIFT-MS protocol used in this study did not adjust for any contribution of ethanol or methanol to reported formaldehyde levels. Unadjusted reported formaldehyde levels in the direct exhaled breath of 34 subjects (25 to 62 years; 11 males; 2 smokers) varied from 0 to 12 ppb with a mean of 2 ppb and a median of 1 ppb (see Table 3-6). Measurements of formaldehyde in exhaled breath condensate ranged from 0 to 12 ppb with a mean of 2 ppb and a median of 0 ppb. All but one measurement was below the average ambient room air concentration of 9.6 ± 1.5 ppb. Although comparisons on the individual level could not be made from the data as reported, the range of ethanol and methanol levels in exhaled breath indicate that 1% of the reported ethanol and methanol may have contributed significantly to the reported formaldehyde levels in exhaled breath (see Table 3-6). It is unclear if the reported formaldehyde may represent in part inhaled formaldehyde, reduced by absorption in the upper respiratory tract, or is an artifact of the reported methanol and ethanol levels.
Table 3-6. Formaldehyde, methanol and ethanol levels reported in the exhaled breath of 34 subjects (25 to 62 years; 11 males; 2 smokers)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Minimum (ppb)</th>
<th>Maximum (ppb)</th>
<th>Mean (ppb)</th>
<th>Median (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>102</td>
<td>2319</td>
<td>297</td>
<td>189</td>
</tr>
<tr>
<td>Ethanol</td>
<td>27</td>
<td>10262</td>
<td>447</td>
<td>82</td>
</tr>
<tr>
<td>1% of the reported levels of both ethanol and methanol</td>
<td>1.3</td>
<td>125</td>
<td>7.3</td>
<td>2.6</td>
</tr>
<tr>
<td>Formaldehyde (tentatively identified with a m/z ratio n = 31)</td>
<td>0</td>
<td>12</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Source: Cáp et al. (2008).

Moser et al. (2005) measured levels of 179 volatile organic compounds (VOCs) in the exhaled breath of 344 individuals. This study was not designed to ascertain whether exhaled formaldehyde is of endogenous origin, but rather to demonstrate that proton transfer reaction-mass spectrometry can be used as a new method for rapid screening of large collectives for risk factors (e.g., smoking behavior), potential disease biomarkers, and ambient air characterization. The study was conducted at a health fair. The test subjects had a mean age of 61.6 years; 63% were males and 14% were smokers. Samples of room air were collected and evaluated in parallel with exhaled breath samples. The authors note that formaldehyde was detected in room air, but did not report the levels; rather they stated that the background concentrations were negligible. Of the 179 volatile organic compounds measured, data were reported for 14, including formaldehyde and formic acid. The report by Moser et al. (2005) does not provide the limit of detection for any of the compounds measured or details of the analytical method. Moser et al. (2005) do note that significant differences in exhaled breath composition could be found between smokers and nonsmokers for 32 of the 179 chemicals measured, but the 32 chemicals were not named and no substantiating data were provided.

The formaldehyde levels in exhaled breath spanned from 1.2 to 72.7 ppb with a median of 4.3 ppb and 75th percentile of 6.3 ppb (see Table 3-7) (Moser et al., 2005). The reported levels of formaldehyde (m/z ratio = 31) we not adjusted for any potential contribution from methanol or ethanol in exhaled breath. The levels of methanol and ethanol in exhaled breath were reported by Moser et al. (2005). Although the summary statistics do not allow comparison...
of individual results, it is possible that reaction fragments from methanol and ethanol may have contributed to the reported formaldehyde levels (see Table 3-7).

Table 3-7. Apparent formaldehyde levels (ppb) in exhaled breath of individuals attending a health fair, adjusted for methanol and ethanol levels which contribute to the detection of the protonated species with a mass to charge ratio of 31 reported as formaldehyde ($m/z = 31$)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Minimum</th>
<th>25th percentile</th>
<th>Median</th>
<th>75th percentile</th>
<th>97.5th percentile</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>13.367</td>
<td>106.227</td>
<td>161.179</td>
<td>243.185</td>
<td>643.614</td>
<td>1536.499</td>
</tr>
<tr>
<td>Ethanol</td>
<td>11.583</td>
<td>23.1</td>
<td>34.664</td>
<td>64.24</td>
<td>549.24</td>
<td>9779.768</td>
</tr>
<tr>
<td>1% of the reported levels of both ethanol and methanol</td>
<td>0.25</td>
<td>1.29</td>
<td>1.96</td>
<td>3.07</td>
<td>11.93</td>
<td>113.16</td>
</tr>
<tr>
<td>Mass of $m/z = 31$ reported as formaldehyde</td>
<td>1.23</td>
<td>3.1</td>
<td>4.26</td>
<td>6.33</td>
<td>39.8</td>
<td>72.7</td>
</tr>
</tbody>
</table>

Source: Moser et al. (2005).

The range of reported formaldehyde is much greater in this study of the general population (attendees at a health fair) than that observed in healthy volunteers discussed above (Wang et al., 2008; Cap et al., 2008; Turner et al., 2008; Kushch et al., 2008). Moser et al. (2005) do not discuss potential causes for this wide range in values, and there was no distinction of the data by sex, age, or health. However, reported formaldehyde in exhaled breath (unadjusted) has been correlated to lung cancer diagnosis with a median of 7.0 ppb and upper 95th CI greater than 30 ppb (Wehinger et al., 2007). Although it is unknown if these results represent only formaldehyde, or are in part an artifact of increased ethanol and methanol in exhaled breath, the higher levels reported by Moser et al. (2005) may reflect volatile levels in unhealthy individuals who attended the public health fair.

Selected ion flow tube mass spectrometry (SIFT-MS), with the recent improvements by Španěl and Smith (2008) to account for the fragments of methanol and ethanol reaction products, have the ability to detect formaldehyde in exhaled breath. However, to date, no data has been published which makes this adjustment for reporting formaldehyde levels. Therefore all of the above reports of formaldehyde in exhaled breath should be carefully interpreted as the mass reported as formaldehyde—is only tentatively identified as formaldehyde. A careful review of the data where methanol and ethanol levels are also provided, indicate that levels of formaldehyde (tentatively identified as $m/z = 31$) may reflect a significant contribution from...
reaction products of methanol and ethanol. In summary, there are insufficient data at this time to confidently establish a concentration of formaldehyde in exhaled breath that can be attributed to endogenous sources. Additional research is needed to further clarify.

3.6.3. Formaldehyde Excretion in Human Urine

Gottschling et al. (1984) examined urinary formic acid in 35 veterinary students. Personal monitoring badges were worn and returned after class, and urine samples were taken prior to class and within 2 hours after the class. Mean exposure levels were about 100 ppb. Baseline averages of urinary formic acid (as a sodium salt) were 12.47 mg/L and ranged from 2.43 to 28.38 mg/L among subjects. Post exposure formate levels were slightly elevated but were not statistically significant. Moreover, formate levels decreased in several individuals relative to pre-exposure levels. The authors concluded that variability in urinary formate may mask any changes and that monitoring formate within 2 hours of exposure is not informative. It is worth noting, however, that interpretation of this finding is confounded due to the fact that diet was not controlled and because no markers for urinary normalization were employed (Boeniger, 1987).

Boeniger (1987) reviewed previously published data on formate in urine (some of which were in German). In one occupational study, workers were exposed to an average formaldehyde exposure of 1.28 mg/m³ over a 6-hour work shift. This implies an average intake of 6 mg;\\(^{6}\) Boeniger reported a range of 2.5 to 13 mg. However, the original study reported that post-shift formate levels were 152 mg/L, whereas the levels were only 24 mg/L 6 days later (no exposure). Considering that only a small percentage of inhaled formaldehyde would be excreted in urine, it is unclear how (or whether) formaldehyde exposure, with the highest total dose of 13 mg, could be responsible for the observed increase.

In the previously described study by Shin et al. (2007), human urine samples were shown to contain TZCA, although variability was not reported. A subsequent study reported that urine TZCA levels were higher in individuals living in newer apartments (0.18 ± 0.121 mg/g creatinine) as compared to older apartments (0.097 ± 0.040 mg/g creatinine) (Li et al., 2007).\\(^{7}\) The authors cited this as evidence that TZCA is a urinary marker for formaldehyde exposure, even though TZCA levels were not correlated to measured (or estimated) formaldehyde exposures. The individuals also differed significantly in age (21.5 vs. 28.6, \(p = 0.053\)) and

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\(^{6}\) 1.28 mg/m³/1,000 L/m³ × 13.8 L/minute × 60 minutes/hour × 6 hours.

\(^{7}\) This study is described in greater detail in Chapter 5.
differed in smoking percentage (10 vs. 27%). Clearly these two studies do not establish a relationship between human formaldehyde exposure and urine TZCA levels.

3.7. MODELING THE TOXICOKINETICS OF FORMALDEHYDE AND DPX

3.7.1. Motivation

Airway geometry is expected to be an important determinant of inhaled formaldehyde dosimetry in the respiratory tract and its differences across species. The uptake of formaldehyde in the upper respiratory tract is highly nonhomogeneous and spatially localized and exhibits strong species differences. Species differences in kinetic factors have been argued to be the key determinants of species-specific lesion distributions for formaldehyde and other reactive inhaled gases. Section 3.7.2 details the benefits to the quantitative risk assessment of modeling these dosimetric differences in the upper respiratory tract. While frank effects were seen only in the upper respiratory tract in rodents, mild lesions were also present in the major bronchiolar region of the rhesus monkey. Therefore, with regard to extrapolation of cancer risk from animal bioassays to humans, it appears that the upper and lower human respiratory tract should both be considered potentially at risk of developing formaldehyde-induced squamous cell carcinoma. Therefore, formaldehyde dose to the lower human respiratory tract also needs to be quantified in order to develop a dose-response relationship that considers the entire respiratory tract.

This assessment uses internal dose metrics computed by using fluid dynamic models to compute regional formaldehyde uptake in the F344 rat and human nasal passages and in the human lower respiratory tract. The assessment also uses estimates of DPX levels in the nasal lining predicted by physiologically-based pharmacokinetic models which use the fluid dynamic model derived estimates of formaldehyde flux to the tissue as input. These computational models enable the derivation of more accurate human equivalent concentrations from the animal bioassays than would be obtained by averaging over the respiratory surface area. The following sections provide the motivation for these calculations, and discuss the strengths and uncertainties associated with the data and the models and their relevance to the hypothesized mode of action are discussed in some length.

3.7.2. Species Differences in Anatomy: Consequences for Gas Transport and Risk

As discussed earlier, formaldehyde is highly reactive and water soluble (categorized as a category 1 gas), thus its absorption in the mucus layer and tissue lining of the upper respiratory tract is known to be significant. The regional inhaled dose of formaldehyde to the respiratory tract of a given species depends on the amount of formaldehyde delivered by inhaled air, the absorption characteristics of the nasal lining, and reactions in the tissue. The amount delivered...
by inhaled air is a function of the major airflow patterns, air-phase diffusion, and absorption at
the airway-epithelial tissue interface. The dose of formaldehyde to the epithelial tissue, which is
different from the amount delivered, depends on the amount absorbed at the airway-tissue
interface, water solubility, mucus-to-tissue phase diffusion, and chemical reactions, such as
hydrolysis, protein binding, and metabolism. It has been argued strongly that species differences
in these kinetic factors are determinants of species-specific lesion distributions for formaldehyde
and other inhaled gases (Moulin et al., 2002; Bogdanffy et al., 1999; Ibanes et al., 1996;
Monticello et al., 1996; Monticello and Morgan, 1994; Morgan et al., 1991).

Because of the convoluted nature of the airways in the upper respiratory tract, the
absorption of such gases in the upper respiratory tract is highly nonhomogeneous. There are
large differences across species in the anatomy of the upper respiratory tract (see Figure 3-6) and
in airflow patterns (see Figure 3-7). Therefore, as shown in the simulations in Figure 3-8, it may
be expected that the uptake patterns, and thus risk due to inhaled formaldehyde, will also show
strong species dependence. Morgan et al. (1991) concluded that airflow-driven dosimetry plays
a critical role in determining the site specificity of various formaldehyde-induced responses,
including tumors, in the nose of the F344 rat. The convoluted geometry of the airway passages
in the upper respiratory tract, as seen from the cross sections of the nose in Figure 3-6, renders an
idealized representation of fluid flow and uptake profiles almost impossible. For these reasons,
Kimbell et al. (1998, 1993), Kepler et al. (1998), and Subramaniam et al. (1998) developed
anatomically realistic finite-element representations of the noses of humans, F344 rats, and
rhesus monkeys. These representations were subsequently used in physical and computational
models (see Figure 3-6). This assessment utilizes dosimetry derived from these representations.

An accurate calculation of species differences in formaldehyde dosimetry in the upper
respiratory tract is important to the extrapolation problem for another reason. The upper
respiratory tract in rats is an extremely efficient scrubber of reactive gases (97% uptake)
(Morgan et al., 1986), thereby protecting the lower respiratory tract from gaseous penetration.
On the other hand, there is considerably more fractional penetration of formaldehyde into the
lower respiratory tract of the rhesus monkey than in the rat (see Figure 3-8). Therefore, an
accurate determination of scrubbing in the upper respiratory tract is important to delineate
species differences in dosimetry in both the upper and lower respiratory tract. Thus, in the case
of the rhesus monkey, the model by Kepler et al. (1998) included the trachea. It is important to
note that the models mentioned above represent nasal passages reconstructed from a single
individual from each species (Kimbell et al., 2001a, b; Conolly et al., 2000; CIIT, 1999;
Subramaniam et al., 1998). This is discussed later in the context of intraspecies variability.
Figure 3-6. Reconstructed nasal passages of F344 rat, rhesus monkey, and human.

Note: Nostril is to the right, and the nasopharynx is to the left. Right side shows the finite element mesh. Left-hand side shows tracings of airways obtained from cross sections of fixed heads (F344 rat and rhesus monkey) and magnetic resonance image sectional scans (humans). Aligned cross sections were connected to form a three-dimensional reconstruction and finite-element computational mesh. Source: Adapted from Kimbell et al. (2001a). Additional images provided courtesy of Dr. J.S. Kimbell, CIIT Hamner Institutes.
Figure 3-7. Illustration of interspecies differences in airflow and verification of CFD simulations with water-dye studies.

Note: Panels A and B show the simulated airflow pattern versus water-dye streams observed experimentally in casts of the nasal passages of rats and monkeys, respectively. Panel C shows the simulated inspiration airflow pattern, and the histogram depicts the simulated axial velocities (white bars) vs. experimental measurements made in hollow molds of the human nasal passages. Dye stream plots were compiled for the rat and monkey over the physiological range of inspiration flow rates. Modeled flow rates in humans were 15 L/minute. Source: Adapted from Kimbell et al. (2001a).
Figure 3-8. Lateral view of nasal wall mass flux of inhaled formaldehyde simulated in the F344 rat, rhesus monkey, and human.

Note: Nostrils are to the right. Simulations were exercised in each species at steady-state inspiration flow rates of 0.576 L/minute in the rat, 4.8 L/minute in the monkey, and 15 L/minute in the human. Flux was contoured over the range from 0–2,000 pmol/(mm²-hour-ppm) in each species.

Source: Kimbell et al. (2001a).

The highly localized nature of uptake patterns shown in Figure 3-8 means that averaging uptake over the entire nasal surface area would dilute the regional dose over areas where response was observed and that an extrapolation based on such averaging would clearly not be accurate.

Another factor to consider in the extrapolation is that monkeys and humans are oronasal breathers while rats are obligate nose-only breathers. Thus, for humans and monkeys, oronasal or oral breathing implies a significantly higher uptake in the lower respiratory tract. It is known...
that a significant fraction of the human population breathes normally through the mouth.

Finally, activity profiles are also determinants of extraction efficiency (see Figure 3-9) and of breathing route (Niinimaa et al., 1981). Given the fact that formaldehyde-induced lesions were observed as far down the respiratory tract as the first bifurcation of the lungs in exposed monkeys, the entire human respiratory tract should be considered when extrapolating data from rats. Thus, for the human, Overton et al. (2001) attached an idealized single-path model of the lower respiratory tract to a model of the upper respiratory tract.

Figure 3-9. CFD simulations of formaldehyde flux to human nasal lining at different inspiratory flow rates.

Note: Right lateral view. Uptake is shown for the nonsquamous portion of the epithelium. The front portion of the nose (vestibule) is lined with keratinized squamous epithelium and is expected to absorb relatively much less formaldehyde.

Source: Kimbell et al. (2001b).
3.7.3. **Modeling Formaldehyde Uptake in Nasal Passages**

Computational models for air flow and formaldehyde uptake in the F344 rat, rhesus monkey, human nose, and human lung were developed by several scientists (Kimbell et al., 1998, 1993; Kepler et al., 1998; Subramaniam et al., 1998; Kimbell et al., 2001a, b). The F344 strain of the rat was chosen since it was assumed to be anatomically representative of its species and because it is widely used experimentally, most notably in bioassays sponsored by the National Toxicology Program. The approximate locations of squamous, mucus-coated, and nonmucus epithelial cells were mapped onto the reconstructed nasal geometry of the computer models. Taken together, these regions of nonmucus and mucus-coated cells comprise the entire surface area of the nasal passages (see original papers and CIIT [1999] for further details on reconstruction and morphometry). Types of nasal epithelium overlaid onto the geometry of the models were assumed to be similar in characteristics across all three species (rat, monkey, and human) except for thickness, surface area, and location. Species-specific mucosal thickness, surface area, and location were estimated from the literature or by direct measurements (Conolly et al., 2000; CIIT, 1999). The nasal passages of all three species were assumed to have a continuous mucus coating over all surfaces except specific areas in the nasal vestibule. As discussed at the beginning of this chapter, formaldehyde hydrolyzes in water and reacts readily with a number of components of nasal mucus. Absorption rates of inhaled formaldehyde by the nasal lining were therefore assumed to depend on where the epithelial lining is coated by mucus and where it is not.

To calculate an airflow rate that would be comparable among species, the amount of inspired air (tidal volume, $V_T$) was divided by the estimated time involved in inhalation (half the time a breath takes, or $(1/2)(1/[breathing frequency, f])$. Thus, an inspiratory flow rate was calculated to be $2V_Tf$, or twice the minute volume. Predicted flux values represent an average of one nasal cycle. Minute volumes were allometrically scaled to 0.288 L/minute for a 315 g rat from data given by Mauderly (1986). Simulations were therefore carried out at 0.576 L/minute for the rat.

The fluid dynamics modeling in the respiratory tract comprises two steps: modeling the airflow through the lumen (solution of Navier-Stokes equations) and modeling formaldehyde uptake by the respiratory tract lining (solution of convective-diffusion equations for a given airflow field). Details of these simulations, including boundary conditions for air flow and mass transfer, are provided in Kimbell et al. (2001a, b; 1998, 1993) and Subramaniam et al. (1998). Formaldehyde absorption at the airway-to-epithelial tissue interface was assumed to be proportional to the air-phase formaldehyde concentration adjacent to the nasal lining layer in
monkeys and humans (see the original paper [Kimbell et al., 2001a, b] for a more detailed elaboration of the calculations for these coefficients).

Because formaldehyde is highly water soluble and reactive, Kimbell at al. (2001a) assumed that absorption occurred only during inspiration. Thus, for each breath, flux into nasal passage walls (rate of mass transport in the direction perpendicular to the nasal wall per mm$^2$ of the wall surface) was assumed to be zero during exhalation, with no backpressure to uptake built up in the tissues. Overton et al. (2001) estimated the error due to this assumption to be small, roughly an underestimate of 3% in comparison to cyclic breathing. Also, this assumption is the same as that used in default methods for reference concentration determination and has been used in other PBPK model applications to describe nasal uptake (Andersen and Jarabek, 2001).

### 3.7.3.1. Flux Bins

A novel contribution of the CIIT biologically motivated dose-response model is that cell division rates and DPX concentrations are driven by the local concentration of formaldehyde. These were determined by partitioning the nasal surface by flux, resulting in 20 “flux bins.” Each bin was comprised of elements (not necessarily contiguous) of the nasal surface that receive a particular interval of formaldehyde flux per ppm of exposure concentration (Kimbell et al., 2001a, b). The spatial coordinates of elements comprising a particular flux bin were fixed for all exposure concentrations, with formaldehyde flux in a bin scaling linearly with exposure concentration (ppm). Thus, formaldehyde flux was expressed as pmol/(mm$^2$-hour-ppm).

### 3.7.3.2. Flux Estimates

Formaldehyde flux was estimated for the rat, monkey, and human over the entire nasal surface and over the portion of the nasal surface that was lined by nonsquamous epithelium. Formaldehyde flux was also estimated for the rat and monkey over the areas where cell proliferation measurements were made (Monticello et al., 1991, 1989) and over the anterior portion of the human nasal passages that is lined by nonsquamous epithelium. Figure 3-8 shows the mass flux of inhaled formaldehyde to the lateral wall of nasal passages in the F344 rat, rhesus monkey, and human (Kimbell et al., 2001a, b).

Maximum flux estimates for the entire upper respiratory tract were located in the mucus-coated squamous epithelium on the dorsal aspect of the dorsal medial meatus near the boundary between nonmucus and mucus-coated squamous epithelium in the rat, at the anterior or rostral margin of the middle turbinate in the monkey, and in the nonsquamous epithelium on the proximal portion of the mid-septum near the boundary between squamous and nonsquamous
epithelium in the human (see Kimbell et al. [2001a, b] for tabulations of comparative estimates of formaldehyde flux across the species).

The rat-to-monkey ratio of the highest site-specific fluxes in the two species was 0.98. In the rat, the incidence of formaldehyde-induced squamous cell carcinomas in chronically exposed animals was high in the anterior lateral meatus (Monticello et al., 1996). Flux predicted per ppm in this site and flux predicted near the anterior or proximal aspect of the inferior turbinate and adjacent lateral walls and septum in the human were similar, with a rat-to-human ratio of 0.84.

3.7.3.3. Mass Balance Errors

Overall uptake of formaldehyde was calculated as 100% × (mass entering nostril − mass exiting outlet)/(mass entering nostril). Mass balance errors for air, 100% × (mass of air entering nostril − mass exiting outlet)/(mass entering nostril), and inhaled formaldehyde, 100% × (mass entering nostril − mass absorbed by airway walls − mass exiting outlet)/(mass entering nostril), were calculated. Mass balance errors associated with simulated formaldehyde uptake from air into tissue ranged from less than 14% for the rat, monkey, and human at 7.4 and 15 L/minute to approximately 27% at the highest inspiratory flow rates of 31.8 and 37 L/minute (Kimbell et al., 2001b). Kimbell et al. (2001b) corrected the simulation results for these errors by evenly distributing the lost mass over the entire nasal surface.

3.7.4. Modeling Formaldehyde Uptake in the Lower Respiratory Tract

Lesions were observed in the lower respiratory tract of rhesus monkeys exposed to 6 ppm formaldehyde. Therefore it is appropriate to consider the human lower respiratory tract as potentially at risk for formaldehyde-induced cancer. Accordingly, fluid flow and formaldehyde uptake in the lower respiratory tract were also modeled for the human in the CIIT approach by using dosimetry estimates for the human lower respiratory tract.

The single-path idealization of the human lung anatomy captures the geometrical characteristics of the airways for a given lung depth, and of airflow through these airways, in an average, homogeneous sense. For particulates, this has provided a reasonable representation of the average deposition in a given generation of the lung airways for a normal human population. The one-dimensional model by Weibel (1963) is generally considered adequate unless the fluid dynamics at airway bifurcations need to be explicitly modeled, and such an idealization of the lung geometry has been successfully used in various models for the dosimetry of ozone and particulate and fibrous matter. Most likely, the lung geometries of the susceptible population, such as those with chronic obstructive pulmonary disease, would depart significantly from the geometry described in Weibel (1963). Unlike the accurate representation of the nasal anatomy
used in the CFD modeling, the lung geometry is idealized in the CIIT approach as a typical path Weibel geometry. The single-path model used to calculate formaldehyde uptake in the human respiratory tract (Overton et al., 2001; CIIT, 1999) applied a one-dimensional equation of mass transport to each generation of an adult human symmetric, bifurcating Weibel-type respiratory tract anatomical model, augmented by an upper respiratory tract. The detailed CFD modeling of the upper respiratory tract was made consistent with the upper respiratory tract in the single-path model by requiring that the one-dimensional version of the nasal passages have the same inspiratory air-flow rate and uptake during inspiration as the CFD simulations for four daily human activity levels. The reader is referred to Overton et al. (2001) for further details of the simulations. Results most relevant to this assessment are shown in Figure 3-10.

Figure 3-10. Single-path model simulations of surface flux per ppm of formaldehyde exposure concentration in an adult male human.

Source: Overton et al. (2001).

The primary predictions of the model, as shown in Figure 3-10, were that more than 95% of the inhaled formaldehyde would be retained and formaldehyde flux in the lower respiratory
tract would increase for several lung airway generations from that in the posterior-most segment of the nose and then decrease rapidly, resulting in almost zero flux to the alveolar sacs.

Overton et al. (2001) modeled uptake at higher inspiratory rates, including those at 50 L/minute of minute volume (well beyond levels where the oronasal switch occurs in the normal nasal breathing population). At these rates Figure 3-8 indicates that formaldehyde flux in the mouth cavity is comparable (but a bit less) to that occurring in the nasal passages. Overton et al. (2001) did not model uptake in the oral cavity at minute volumes less than 50 L/minute. This would be of interest because mouth breathers form a large segment of the population. Furthermore, at concentrations of formaldehyde where either odor or sensory irritation becomes a significant factor, humans are likely to switch to mouth breathing even at resting inspiration. At a minute volume of 50 L/minute, Overton et al. (2001) assumed, citing Niinimaa et al. (1981), that 0.55 of the inspired fraction is through the mouth. Therefore, based on the results in Figure 3-8, it is not unreasonable to assume that for mouth breathing conditions at resting or light exercise inspiratory rates, average flux across the human mouth lining would be comparable to the average flux across the nasal lining computed in Kimbell et al. (2001a, b).

3.7.5. Uncertainties in Formaldehyde Dosimetry Modeling

3.7.5.1. Verification of Predicted Flow Profiles

The simulated streamlines of steady-state inspiration airflow predicted by the CFD model agreed reasonably well with experimentally observed patterns of water-dye streams made in casts of the nasal passages for the rat and monkey as shown in panels A and B in Figure 3-7. The airflow velocity predicted by CFD model simulations of the human also agreed well with measurements taken in hollow molds of the human nasal passages (panel C, Figure 3-8) (Kepler et al., 1998; Subramaniam et al., 1998; Kimbell et al., 1997a, 1998, 1993). However, the accuracy and relevance of these comparisons are limited. The profiles were verified by video analysis of dye streak lines in the molds of rats and rhesus monkeys, although this method is reasonable for only the major airflow streams.

Plots of pressure drop vs. volumetric airflow rate predicted by the CFD simulations compared well with measurements made in rats in vivo (Gerde et al., 1991) and in acrylic casts of the rat nasal airways (Cheng et al., 1990) as shown in Figure 3-11. This latter comparison remains qualitative due to differences among the simulation and experiments as to where the outlet pressure was measured and because no tubing attachments or other experimental apparatus were included in the simulation geometry. The simulated pressure drop values were somewhat lower, possibly due to these differences.
Figure 3-11. Pressure drop vs. volumetric airflow rate predicted by the CIIT CFD model compared with pressure drop measurements made in two hollow molds (C1 and C2) of the rat nasal passage (Cheng et al., 1990) or in rats in vivo (Gerde et al., 1991).

Source: Kimbell et al. (1997a).

Inspiratory airflow was assumed to be constant in time (steady state). Subramaniam et al. (1998) considered this to be a reasonable assumption during resting breathing conditions based on a value of 0.02 obtained for the Strouhal number. Unsteady effects are insignificant when this number is much less than one. However, this assumption may not be reasonable for light and heavy exercise breathing scenarios.

3.7.5.2. Level of Confidence in Formaldehyde Uptake Simulations

Unlike the airflow simulations, it was not possible to evaluate the formaldehyde uptake calculations directly. Since the mass transfer boundary conditions were set by fitting overall uptake to the average experimental data for various exposure concentrations, it was not possible to independently verify even the overall uptake values with empirical data. This assessment has relied on several indirect qualitative and quantitative lines of evidence listed below to provide general confidence in the uptake profile for the F344 rat nasal passages, as modeled in CIIT (1999), when gross averages are considered over certain regions of the nasal lining.
In an earlier simulation, where the nasal walls were set to be infinitely absorbing of formaldehyde, uptake of inhaled formaldehyde in the upper respiratory tract was predicted to be 90% in the rat for simulations corresponding to the resting minute volume in the F344 rat. This estimate compared reasonably well with the range of 91–98% observed by Morgan et al. (1986a).

Morgan et al. (1991) showed general qualitative correspondence between the main routes of flow and lesion distribution induced by formaldehyde in the rat nose. In their initial work with a CFD model that represented a highly reactive and soluble gas, Kimbell et al. (1998, 1993) described similarities in computed regional mass flux patterns and lesion distribution due to formaldehyde. When the results from this work in the coronal section immediately posterior to the vestibular region were considered, simulated flux levels over regions such as the medial aspect of the maxilloturbinate and the adjacent septum (where lesions were seen) were an order of magnitude higher than over other regions, such as the nasoturbinate (where lesions were not seen).^8

The results of a PBPK model by Cohen-Hubal et al. (1997) provide a reasonable level of confidence in regional uptake simulations for the F344 rat when gross averages over nasal sites are carried out. Cohen-Hubal et al. (1997) linked the CFD dosimetry model for formaldehyde to a PBPK model for formaldehyde-DPX concentration in the F344 rat. This PBPK model was calibrated by optimizing the model to combined DPX data from all regions of the rat nose (high-tumor and low-tumor incidence regions) that were obtained in separate experiments by Casanova et al. (1991, 1989). These data were obtained at 0.3, 0.7, 2.0, 6.0, and 10 ppm for both regions. DPX data were also obtained at 15 ppm exposure from the high-tumor region; however these were not included for the calibration. Model prediction of DPX concentrations were then compared with data for the high-tumor region only and compared well with the experimental data, including the 15 ppm data for which the model had not been calibrated. This is shown in Figure 3-12. Such a verification, albeit indirect, is not available for the simulation of uptake patterns in the human.

The CFD simulations do not model reflex bradypnea, a protective reflex seen in rodents but not in humans. As discussed at length in Sections 3.2.3.1 and 4.2.1.1, it is reasonable to

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^8 However, this 1993 CFD model differed somewhat from the subsequent model by Kimbell et al. (2001a) used in this assessment. In the 1993 model, the limiting mass-transfer resistance for the gas was assumed to be in the air phase; that is, the concentration of formaldehyde was set to zero at the airway lining. Furthermore, this same boundary condition was used on the nasal vestibule as well, while, in the more recent model, the vestibule was considered to be nonabsorbing. Unfortunately, Kimbell at al. (2001a) did not report on correspondences between flux patterns and lesion distribution.
Figure 3-12. Formaldehyde-DPX dosimetry in the F344 rat.

Panel A: calibration of the PBPK model using data from high and low tumor incidence sites. Panel B: model prediction compared against data from high tumor incidence site. Dashed line in panel A shows the extrapolation outside the range of the calibrated data.

Source: Cohen-Hubal et al. (1997).

1 expect a range of 25% (Chang et al., 1983) to 45% (Barrow et al., 1983) decrease in minute volume in F344 rats at the exposure concentration of 15 ppm. Explicit omission of this effect in the modeling is, however, not likely to be a source of major uncertainty in the modeled results.

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for uptake of formaldehyde in the rat nose for the following reason. The CFD model for the
F344 rat was calibrated to fit the overall experimental result for formaldehyde uptake in the F344
rat at 15 ppm exposure concentration. This was carried out by adjusting the mass transfer
coefficient used as boundary condition on the absorbing portion of the nasal lining. Thus, the
reflex bradypnea occurring in those experimental animals is phenomenologically factored into
the value used for the boundary condition. Nonetheless, some error in the localized distribution
of uptake patterns may be expected, even if the overall uptake is reproduced correctly.
Furthermore, since the same value for the mass transfer coefficient was used in human
simulations (as obtained from calibration of the rat model), there is additional uncertainty in the
modeled human flux estimates. This issue was not addressed by Kimbell et al. (2001a, b),
Conolly et al. (2004), or Schlosser et al. (2003), and we are unable to assess the extent of this
error more accurately.

3.7.6. PBPK Modeling of DNA Protein Cross-Links (DPXs) Formed by Formaldehyde

3.7.6.1. PBPK Models for DPXs

As can be seen from the previous sections, measuring the distribution of the absorbed
formaldehyde and identifying its form have proven difficult. Because of the high reactivity of
formaldehyde, rapid metabolism of formaldehyde, and complexity of formate clearance, dose
surrogates (or biomarkers) of exposure have been used to characterize the extent of absorption
and distribution of formaldehyde. As with other soluble and reactive gases, typical PBPK
models that predict steady-state blood concentrations are not useful for predicting formaldehyde
dosimetry at this time. As noted previously, inhalation exposure to formaldehyde has not been
shown to increase blood formaldehyde levels. Thus, most modeling efforts for formaldehyde
have focused on disposition at the site of contact.

As discussed earlier, the concentration of DPXs formed by formaldehyde has been
treated as a surrogate for the tissue dose of formaldehyde in earlier efforts by Casanova et al.
(1991) and in EPA’s efforts to update its health assessment of formaldehyde (Hernandez et al.,
1994). These efforts used data from rats and rhesus monkeys (Casanova et al., 1991, 1989).
Using DPXs in this manner allowed the incorporation of both clearance and metabolism of
formaldehyde and the incorporation of the effect of saturation on detoxification of formaldehyde
at higher doses. Calculation of the average DPX concentration from these data was seen as a
surrogate for the area under the curve (AUC) of the reactive formaldehyde species in the
epithelium. Based on these data, Casanova et al. (1991) developed a PBPK model for predicting
DPXs in these species and for extrapolating to the human.
The Casanova et al. (1991) model consists of three anatomical compartments representing different parts of the upper respiratory tract of the rhesus monkey. The results indicated a 10-fold difference in DPX formation between rats and monkeys, due primarily to species differences in minute volume and differing quantities of DNA in the nasal mucosa. Casanova et al. (1991) then developed a monkey/rat scaling factor for these parameters by taking the ratio of nasal mucosa tissue between the two species, a determinant that was proportional to the total body weight differences between the two species. Using these scaling factors in their model, the authors’ predictions in monkey (based on the rat data) were in close agreement with observed DPXs in monkey, particularly at higher formaldehyde concentrations. However, the model overpredicted DPX formation in the monkey at lower formaldehyde concentrations. Subsequent rat-human and monkey-human scaling results predicted much lower DPX formation in man. Again, the values obtained at lower concentrations may have been overpredicted, as was the case for the rat-monkey extrapolation.

Georgieva et al. (2003) developed a model for the uptake and disposition of formaldehyde in the rat nasal lining. This model was designed to predict the distribution of formaldehyde in the nasal mucosa. The model indicated that, at 6 ppm exposure, a steady-state elevation of 15–20 µM formaldehyde would be achieved within 30 seconds. Furthermore, this same elevation was predicted when the exposure was 6 ppm formaldehyde for 60 minutes. Given that human blood formaldehyde levels are predicted to be about 100 ± 15 µM (Heck et al., 1985) and assuming that blood formaldehyde concentration is roughly equivalent to the concentration predicted at the basement membrane of the epithelium, this model predicts roughly a 15–20% increase in blood formaldehyde. However, it should be noted that a 40-minute inhalation exposure of humans to 1.99 ppm formaldehyde did not lead to a measurable increase in blood formaldehyde (Heck et al., 1985).

Franks (2005) published a mathematical model for predicting the disposition of formaldehyde in the human nasal mucosa and blood. The calculated concentrations of formaldehyde in the mucus, the epithelium, and the blood attained steady-state profiles within a few seconds of exposure. The increase of the formaldehyde concentration in the blood was predicted to be insignificant compared with the existing pre-exposure levels in the body: an increase of 0.00044 mg/L in blood formaldehyde following exposure to 1.9 ppm formaldehyde for up to 8 hours. The model described formaldehyde concentration gradients across the mucus, epithelial, and submucosal compartments in the human nose. Transport of formaldehyde was governed by the following processes: diffusional (in the mucus); a combination of diffusional, two first order terms representing intrinsic reactivity of formaldehyde and binding to DNA, and Michaelis-Menten kinetics representing enzymatic metabolism (in the epithelial layer); a first-
order term representing nonenzymatic removal governed by the blood perfusion rate (in the submucosal compartment). The model used the values for the first order reaction rate constants and the Michaelis-Menten parameters ($V_{\text{max}}$ and $K_m$) estimated by Conolly et al. (2000) in their model for extrapolating the rat and rhesus monkey data to the human. The modeling in Franks (2005) was not calibrated or validated against experimental data, but the predictions of negligible penetration of free formaldehyde to the blood are qualitatively in agreement with the conclusions in Heck et al. (1985).

Following the efforts by Casanova and coworkers, Cohen-Hubal et al. (1997), Conolly et al. (2000), and Georgieva et al. (2003) developed models that linked local formaldehyde flux from CFD models to DPX predictions. The focus here will be on the Conolly et al. (2000) effort for the following two reasons: it explicitly incorporates regional formaldehyde dosimetry in the nasal lining by using results from CFD modeling of airflow and gas uptake and it brings data across species (rat and rhesus monkey) to bear on model calibration, such a situation being relatively rare in chemical health risk assessments.

3.7.6.2. A PBPK Model for DPXs in the F344 Rat and Rhesus Monkey that Uses Local Tissue Dose of Formaldehyde

In earlier risk assessment efforts (Hernandez et al., 1994; Casanova et al., 1991; U.S. EPA, 1991b), the average DPX concentration was considered a surrogate tissue dose metric for the AUC of the reactive formaldehyde species. Conolly et al. (2000) assigned a more specific role for DPXs, treating local DPX concentration as a dose surrogate indicative of the intercellular concentration of formaldehyde, leading to formaldehyde-induced mutations. These authors indicated that it was not known whether DPXs directly induced mutations (Conolly et al., 2003; Merk and Speit, 1998). This is discussed in detail in the mode-of-action sections in this document. The Conolly et al. (2000) model for the disposition of inhaled formaldehyde gas and DPX in the rat and rhesus nasal lining is relatively simple in terms of model structure because it consists of a single well-mixed compartment for the nasal lining as follows:

1. Formaldehyde flux to a given region of the nasal lining is provided as input to the modeling and is obtained in turn as the result of a CFD model. This flux is defined as the amount of formaldehyde delivered to the nasal lining per unit time per unit area per ppm of concentration in the air in a direction transverse to the airflow. It is locally defined as a function of location in the nose and the inspiratory flow rate and is linear with exposure concentration.

2. The clearance of formaldehyde from the tissue is modeled as follows:
a. a saturable pathway representing enzymatic metabolism of formaldehyde, which is primarily by formaldehyde dehydrogenase (involving Michaelis-Menten parameters $V_{\text{max}}$ and $K_m$)

b. a separate first-order pathway, which is assumed to represent the intrinsic reactivity of formaldehyde with tissue constituents (rate constant $k_f$)

c. first-order binding to DNA that leads to DPX formation (rate constant $k_b$)

3. The clearance or repair of this DPX is modeled as a first order process (rate constant $k_{\text{loss}}$).

**DPX data.** DPX concentrations were estimated from a study by Casanova et al. (1994) in which rats were exposed 6 hours/day, 5 days/week, plus 4 days for 11 weeks to filtered air (naive) or to 0.7, 2, 6, or 15 ppm (0.9, 2.5, 7.4, or 18 mg/m³) formaldehyde (pre-exposed). On the 5th day of the 12th week, the rats were then exposed for 3 hours to 0, 0.7, 2, 6, or 15 ppm $^{14}$C-labeled formaldehyde (with pre-exposed animals exposed to the same concentration as during the preceding 12 weeks and 4 days). The animals were sacrificed and DPX concentrations determined at two sites in the nasal mucosa. Conolly et al. (2000) used these naive rat data to develop a PBPK model that predicted the time-course of DPX concentrations as a function of formaldehyde flux at these sites.9

**3.7.6.3. Uncertainties in Modeling the Rat and Rhesus DPX Data**

**3.6.6.3.1. Half-life of DPX repair.** In the development of the PBPK model for DPXs, Conolly et al. (2000) assumed a value of $6.5 \times 10^{-3}$ minute$^{-1}$ for $k_{\text{loss}}$, the first-order rate constant for the clearance (repair) of DPXs, such that the DPXs predicted at the end of a 6-hour exposure to 15 ppm were reduced to exactly the detection limit for DPXs in 18 hours (the period between the end of 1 day’s 6-hour exposure and the beginning of the next). This determination of rapid clearance was based on an observation by Casanova et al. (1994) that the DPX concentrations observed in the pre-exposed animals were not significantly higher than those in naïve animals (in which there was no significant DPX accumulation). However, in vitro data (Quievryn and Zhitkovich, 2000) indicate a much slower clearance, with an average $k_{\text{loss}}$ of $9.24 \times 10^{-4}$ minute$^{-1}$.

Subramaniam et al. (2007) examined the Casanova et al. (1994) data and argued that there was a significantly decreased (~ 40%) level of DPXs in high tumor regions of pre-exposed animals vs. naive animals at 6 and 15 ppm and that the weight of the tissues dissected from those animals was also significantly decreased (~ 30%).

9 Subramaniam et al. (2007) who also used the same data verified that they were on naïve rats; however, Conolly et
regions increased substantially, indicating a thickening of the tissues. After testing the outcome of changing the tissue thickness in the PBPK model for DPXs, it was apparent to these authors that such a change alone could not account for the dramatic reduction in DPX levels after pre-exposure, even with the higher value of $k_{\text{loss}}$ used by Conolly et al. (2000). Therefore, in addition to the gross increase in tissue weight, these data indicated either an induction in the activity of enzymes that remove formaldehyde (aldehyde and formaldehyde dehydrogenase) or other changes in the biochemical properties of the highly exposed tissue that must have occurred. Given such a change, Subramaniam et al. (2007) concluded that the experimental results in Casanova et al. (1994) were consistent with the smaller experimental value of $k_{\text{loss}}$ indicated by the Quievryn and Zhitkovich (2000) data. In particular, they argued that if $V_{\text{max}}$ increased with exposure (in a tissue region- and dose-specific manner), then it was possible to explain the naïve vs. pre-exposed data of Casanova et al. (1994), with the value of $k_{\text{loss}}$ effectively measured in vitro by Quievryn and Zhitkovich (2000). Furthermore, this value was measured directly, rather than obtained by indirect interpretation of measurements made at only two time points where significant changes in the tissue had occurred. Therefore, Subramaniam et al. (2007) considered the use of this lower value for $k_{\text{loss}}$ to be more appropriate. The same lower value of $k_{\text{loss}}$ was also used by Georgieva et al. (2003). Consequently, they reimplemented and reoptimized the Conolly et al. (2000) model with this modification and found that the fit so obtained to the acute DPX data was excellent. The reimplemented model will be used in this assessment, and more details can be found in Subramaniam et al. (2007).

It should be noted that this slower DPX repair rate was obtained in an in vitro study by using human cell lines that were transformed and immortalized. However, it appears that DPX repair in normal cells would be even slower. When nontransformed freshly purified human peripheral lymphocytes were used instead, the half-life for DPX repair was about 50% longer than in the cultured cells (Quievryn and Zhitkovich, 2000).

### 3.6.6.3.2. Statistical uncertainty in parameter estimates and extrapolation.

Klein et al. (2010) developed methods for deriving statistical inferences of results from PBPK models, and used the structure of the Conolly et al. (2000) model for demonstrating their methods, specifically because of the sparse time-course information in the above DPX data. However, they used the value of $k_{\text{loss}}$ deduced from Quievryn and Zhitkovich (2000) and fitted the model simultaneously to both the rat and rhesus monkey data, as opposed to the sequential fitting in Conolly et al. (2000). They found that the predicted DPX concentrations were extremely sensitive to $V_{\text{max}}$ and

al. (2000) state that they used data on pre-exposed rats.
tissue thickness as was also concluded by Georgieva et al. (2003) and Cohen-Hubal et al. (1997). 
K_m was seen to be substantially different across species, a finding that was attributed plausibly 
to the involvement of more than one enzyme (Klein et al., 2010; Georgieva et al., 2003). Klein 
et al. (2010) concluded that the two efforts (Conolly et al. [2000] vs. Klein et al. [2010]) resulted 
in substantially different predictions outside the range of the observed data over which the 
models were calibrated.

The differences between these models occur in spite of the fact that both methods use all 
the available DPX data in both species and the same model structures. At the 0.1 ppm exposure 
concentration, in general these authors obtained three- to fourfold higher DPX concentrations 
averaged over a 24-hour period after exposure. Furthermore, the standard deviations in Klein et 
al. (2010) for V_max and K_m were an order of magnitude higher and that for k_f was 35-fold lower 
than the corresponding standard deviations reported in Conolly et al. (2000). The relatively 
larger standard deviation for k_f resulted in this parameter becoming negative in Conolly et al. 
(2000) at half the standard deviation below the maximum likelihood estimate (MLE) value. Note 
that, at a negative value of k_f, formaldehyde would be produced as opposed to being cleared 
through its intrinsic reactivity.

Klein et al. (2010) concluded that these “remarkable differences outside the range of the 
observed data suggest caution in the use of these models in a predictive sense for extrapolating to 
human exposures.”

3.7.7. Uncertainty in Prediction of Human DPX Concentrations

Conolly et al. (2000) used both the rat and rhesus monkey data to predict human DPX 
concentrations and constructed a PBPK model for the rhesus monkey along similar lines as for 
the F344 rat. In the rhesus monkey model, they maintained the same values of k_b, k_loss, and k_f as 
in the rat model but optimized the values of V_max and K_m against the rhesus monkey data from 
Casanova et al. (1994). The rat and rhesus monkey parameters were then used to construct a 
human model (see Conolly et al. [2000] for a more detailed report of implementing the rhesus 
monkey model and the extrapolating to humans).

For the human, the model used the value of K_m obtained in the rhesus monkey model and 
the epithelial thickness averaged over three regions of the rhesus monkey nose. The maximum 
rate of metabolism, V_max, which was estimated independently for the rat and rhesus monkey by 
fitting to the DPX data available for these species, was then extrapolated to the human by 
assuming a power law scaling with body weight (BW) (i.e., V_max = a × BW^b), and the coefficient 
“a” and exponent “b” were derived from the independently estimated values of (V_max)RAT and
Table 3-8. Extrapolation of parameters for enzymatic metabolism to the human

<table>
<thead>
<tr>
<th>Parameter</th>
<th>F344 rat</th>
<th>Rhesus monkey</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{max}}$ (pmol/min-mm$^3$)</td>
<td>1,008.0</td>
<td>91.0</td>
<td>15.7</td>
</tr>
<tr>
<td>$K_{\text{m}}$ (pmol/mm$^3$)</td>
<td>70.8</td>
<td>6.69</td>
<td>6.69</td>
</tr>
</tbody>
</table>

Source: Conolly et al. (2000).

The above scale-up procedure was an attempt to use both the rodent and primate DPX data. However, laws for allometric scaling across species, such as how enzymatic metabolic rates vary across organisms, are empirical regression relationships whose strength is that they are based on data from multiple species and usually multiple sources of data points. For example, West and Brown (2005) demonstrate that metabolic rates scale with mass$^{3/4}$ using data from organisms ranging over 27 orders of magnitude in mass (intracellular up to the largest organisms). In Conolly et al. (2000) the power-law relationship is derived using two data points (F344 rat and rhesus monkey for a single chemical) with log BW as x-axis and $V_{\text{max}}$ on y-axis. Since such a regression does not have the power to delineate the curvature in the scaling function, the empirical strength of the allometric relationship derived in Conolly et al. (2000) is extremely weak for use in extrapolating from the rat to the human on the basis of body-weight.

The following observations point to the uncertainty in the values of the parameters $V_{\text{max}}$ and $K_{\text{m}}$ in the Conolly et al. (2000) models for predicting DPXs. First, $K_{\text{m}}$ varies by an order of magnitude across the rat and monkey models but is then considered invariant between the monkey and human models (Conolly et al., 2000). Second, the values in Conolly et al. (2000) for $V_{\text{max}}/K_{\text{m}}$, the low-dose limit of the rate of enzymatic metabolism, is roughly similar between the rat and monkey but lower by a factor of six in the human.

Another factor that can substantially influence the above extrapolation of DPXs in the human is that Conolly et al. (2000) assumed the tissue to be a well-mixed compartment with regard to formaldehyde interaction with DNA and used the amount of formaldehyde bound to DNA per unit volume of tissue as the DPX dose metric. Considering formaldehyde’s highly reactive nature, the concentrations of formaldehyde and DPX are likely to have a sharp gradient with distance into the nasal mucosa (Georgieva et al., 2003). Given the interspecies differences in tissue thickness, there is consequent uncertainty as to whether DPX per unit volume or DPX
per unit area of nasal lining is the more appropriate dose metric to be used in the extrapolation. In particular, it may be assumed that the cells at risk for tumor formation are only those in the epithelium and that measured DPX data (in monkeys and rats) are an average over the entire tissue thickness. Since the epithelial DPXs in monkeys (and presumably humans) would then be more greatly “diluted” by lower levels of DPX formation that occur deeper into the tissue than in rats, it could be predicted that the ratio of epithelial to measured DPXs in monkeys and humans would be much higher than the ratio in rats.

3.7.8. Modeling Interindividual Variability in the Nasal Dosimetry of Reactive and Soluble Gases

Garcia et al. (2009) used computational fluid dynamics to study human variability in the nasal dosimetry of reactive, water-soluble gases in 5 adults and 2 children, aged 7 and 8 years old. The sample size in this study is too small to consider the results representative of the population as a whole (as also recognized by the authors). Nonetheless, various comparisons with the characteristics of other study populations add to the strength of this study (see Appendix B). The authors considered two model categories of gases, corresponding to maximal and moderate absorption at the nasal lining. We focus here only on the “maximum uptake” simulations in Garcia et al. (2009). In this case, the gas was considered so highly reactive and soluble that it was reasonable to assume an infinitely fast reaction of the absorbed gas with compounds in the airway lining. Although such a gas could be reasonably considered as a proxy for formaldehyde, these results cannot be fully utilized to inform quantitative estimates of formaldehyde dosimetry (and it does not appear to have been the intent of the authors either). This is because the same boundary condition corresponding to maximal uptake was applied on the vestibular lining of the nose as well as on the respiratory and transitional epithelial lining on the rest of the nose. This is not appropriate for formaldehyde as the lining on the nasal vestibule is made of keratinized epithelium which is considerably less absorbing than the rest of the nose (Kimbell et al., 2001b).

The Garcia et al. (2009) study and the results of their analyses have been further described and evaluated in Appendix B. Overall uptake efficiency, average flux (rate of gas absorbed per unit surface area of the nasal lining) and maximum flux levels over the entire nasal lining did not vary substantially between adults (1.6-fold difference in average flux and much less in maximum flux), and the mean values of these quantities were comparable between adults and children. These results are also in agreement with conclusions reached by Ginsberg et al. (2005) that overall extrathoracic absorption of highly and moderately reactive and soluble gases (corresponding to Category 1 and 2 reactive gases as per the scheme in EPA [1994]) is similar in
adults and children. On the other hand, Figure 6A of the paper (reproduced as Figure B-1 in Appendix B), provides a different perspective on variations between the adults in flux values at specific points on the nasal walls. The plot indicates that local flux of formaldehyde may vary among individuals by a factor of 3 to 5 at various distances along the septal axis of the nose; such an evaluation of inter-individual variability in the spatial distribution of formaldehyde flux over the nasal lining is important for a highly reactive and soluble gas whose regional absorption is highly nonhomogeneously distributed (see text surrounding Figure 3-8).
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