

process. The Agency consulted with the SAB in October 1999 (USEPA 2000f).

The Stage 1 DBPR MCLG of zero for chloroform was challenged, and the U.S. Court of Appeals for the District of Columbia Circuit issued an order vacating the zero MCLG (*Chlorine Chemistry Council and Chemical Manufacturers Association v. EPA*, 206 f.3d 1286 (D.C. Circuit 2000)). EPA committed to the Court to propose a non-zero MCLG for chloroform in the upcoming proposed Stage 2 Disinfectants and Disinfection Byproducts Rule. EPA removed the MCLG for chloroform from its Stage 1 DBP NPDWR (USEPA 2000e). No other provision of the Stage 1 DBPR was affected.

b. *Basis of the new chloroform MCLG.* Based on an analysis of all the available scientific data on chloroform discussed in more detail below, EPA believes that chloroform dose-response is nonlinear and that chloroform is likely to be carcinogenic only under high exposure conditions. EPA's assessment of the cancer risk associated with chloroform exposure (USEPA 2001b) uses the principles of the 1999 EPA Proposed Guidelines for Carcinogen Risk Assessment (USEPA 1999a).

The Proposed Guidelines for Carcinogen Risk Assessment, as reviewed by the public and the EPA SAB, reflect new science and are consistent with, and an extension of, the existing 1986 Guidelines for Carcinogen Risk Assessment (USEPA 1986). The 1986 guidelines provide for departures from default assumptions such as low dose linear extrapolation. For example, the 1986 EPA guidelines reflect the position of the Office of Science and Technology Policy (OSTP) that (OSTP 1985; Principle 26) "[N]o single mathematical procedure is recognized as the most appropriate for low-dose extrapolation in carcinogenesis. When relevant biological evidence on mechanisms of action exists (e.g., pharmacokinetics, target organ dose), the models or procedure employed should be consistent with the evidence." The 1985 guidelines go on to state "The Agency will review each assessment as to the evidence on carcinogenesis mechanisms and other biological or statistical evidence that indicates the suitability of a particular extrapolation model."

i. *Mode of action.* EPA has fully evaluated the science on chloroform and concludes that chloroform is likely to be carcinogenic to humans under high exposure conditions that lead to cytotoxicity and regenerative hyperplasia in susceptible tissue; chloroform is not likely to be

carcinogenic to humans at a dose level that does not cause cytotoxicity and cell regeneration (USEPA 1998e, USEPA 1998b, USEPA 2001b).

Chloroform's carcinogenic potential is indicated by animal tumor evidence (liver tumors in mice and renal tumors in both mice and rats) from inhalation and oral exposure. Data on metabolism, toxicity, mutagenicity and cellular proliferation contribute to an understanding of the mode of carcinogenic action. For chloroform, sustained or repeated cytotoxicity with secondary regenerative hyperplasia precedes, and is a key event for, hepatic and renal neoplasia.

EPA believes that a DNA reactive mutagenic mode of action is not likely to be the predominant influence of chloroform on the carcinogenic process. EPA has concluded that the predominant mode of action involves cytotoxicity produced by the oxidative generation of highly reactive metabolites, followed by regenerative cell proliferation (USEPA 2001b). EPA further believes that the chloroform dose-response is nonlinear. The SAB final report states "(t)he Subcommittee agrees with EPA that sustained or repeated cytotoxicity with secondary regenerative hyperplasia in the liver and/or kidney of rats and mice precedes, and is probably a causal factor for, hepatic and renal neoplasia" (USEPA 2000f).

ii. *Metabolism.* The cytochrome P450 isoenzyme CYP 2E1 is the primary enzyme catalyzing chloroform metabolism at low concentrations. Chloroform's carcinogenic effects involve oxidative generation of reactive and toxic metabolites (phosgene and hydrochloric acid [HCl]) and thus are related to its noncancer toxicities (e.g., liver or kidney toxicities). The electrophilic metabolite phosgene could react with macromolecules such as phosphatidyl inositols or tyrosine kinases which in turn could potentially lead to interference with signal transduction pathways (i.e., chemical messages controlling cell division), thus leading to carcinogenesis. Likewise, it is also plausible that phosgene reacts with cellular phospholipids, peptides and proteins resulting in generalized tissue injury. Glutathione, free cysteine, histidine, methionine and tyrosine are all potential reactants for electrophilic agents.

At high concentrations, chloroform may undergo reductive metabolism which forms reactive dichloromethyl free radicals. These free radicals can contribute to lipid peroxidation and cause cytotoxicity.

c. *How the MCLG is derived.* EPA continues to recognize the strength of the science in support of a nonlinear approach for estimating the carcinogenicity of chloroform. This science was affirmed by the Chloroform Risk Assessment Review Subcommittee of the EPA SAB Executive Committee which met on October 27–28, 1999 (USEPA 2000f). The SAB Subcommittee agreed that the nonlinear approach is most appropriate for the risk assessment of chloroform.

Nonzero MCLGs are scientifically and statutorily supported. The statute requires that the MCLG be set where no known or anticipated adverse effects occur, allowing for an adequate margin of safety (56 FR 3533; USEPA 1991b). Historically, EPA established MCLGs of zero for known or probable human carcinogens based on the principle that any exposure to carcinogens might represent some finite level of risk. If there is substantial scientific evidence, however, that indicates there is a "safe threshold", then a nonzero MCLG can be established with an adequate margin of safety (56 FR 3533; USEPA 1991a)).

EPA would ideally like to use the delivered dose (i.e., the amount of key chloroform metabolites that actually reach the liver and cause cell toxicity) for calculating an RfD to support the MCLG. However, the required toxicokinetic data are not currently available. Thus, the RfD is calculated using the applied dose (i.e., the amount of chloroform ingested). The RfD is based on both the benchmark dose and the traditional no observed adverse effect level/lowest observed adverse effect level (NOAEL/LOAEL) approaches for hepatotoxicity in the most sensitive species, the dog. The MCLG is based on the RfD and calculated as follows:

$$\text{MCLG} = \frac{\text{RfD} \times \text{body weight} \times \text{RSC}}{\text{daily water consumption}}$$

i. *Reference dose.* The RfD for chloroform was estimated based on noncancer effects using both the benchmark dose and the traditional NOAEL/LOAEL approaches. For benchmark analysis, five relevant data sets including target organ toxicity, labeling index, histopathology in rodents, and liver toxicity in dogs (Heywood 1979) were evaluated. The effects seen in dogs are considered to be early signs of liver toxicity, preceding cytotoxicity, cytolethality and regenerative hyperplasia. Thus, the Heywood (1979) study, provides the most sensitive end point in the most sensitive species and is the most appropriate basis for the RfD.