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Review

A review of the genotoxicity of 1,2-dichloroethane (EDC)

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ABSTRACT

1,2-Dichloroethane (EDC, CAS#107-06-2) is a high production volume halogenated aliphatic hydrocarbon that is used mainly in the manufacture of vinyl chloride. EDC has been found in ambient and residential air samples, as well as in groundwater, surface water and drinking water. EDC has been well-studied in a variety of genotoxicity assays, and appears to involve the metabolic activation of the parent compound. We critically evaluated the genotoxicity data of EDC and its metabolites as part of an evaluation of carcinogenic mechanisms of action of EDC. EDC is genotoxic in multiple test systems via multiple routes of exposure. EDC has been shown to induce DNA adduct formation, gene mutations and chromosomal aberrations in the presence of key activation enzymes (including CYP450s and/or GSTs) in laboratory animal and *in vitro* studies. EDC was negative for clastogenesis as measured by the micronucleus assay in mice. In general, an increased level of DNA damage is observed related to the GSH-dependent bioactivation of EDC. Increased chromosomal aberrations with increased CYP450 expression were suggestive of a role for the oxidative metabolites of EDC in inducing chromosomal damage. Taken together, these studies demonstrate that EDC exposure, in the presence of key enzymes (including CYP450s and/or GSTs), leads to DNA adduct formation, gene mutations and chromosomal aberrations.
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1. Introduction

1,2-Dichloroethane (EDC, CAS#107-06-2) is a high production volume halogenated aliphatic hydrocarbon that has been found in ambient and residential air samples, as well as in groundwater, surface water and drinking water [1,2]. Used mainly in the manufacture of vinyl chloride, EDC has also been utilized in the

Abbreviations: CYP450, cytochrome P450; EDC, 1,2-dichloroethane; GSH, glutathione; GST, glutathione-S-transferase.

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production of 1,1,1-trichloroethane, trichloroethylene and perchloroethylene. Other uses have included scavenging lead in gasoline, cleaning textiles and equipment, extracting oil from seeds, processing animal fats, pesticides and pharmaceuticals [3]. EDC is also produced as a principal byproduct in the reaction of ethylene and hypochlorous acid, a process used to manufacture ethylene chlorohydrin, which is used, in turn, to produce ethylene oxide [4,5].

Epidemiologic studies have indicated the potential association of exposure to various chemical mixtures containing EDC with human carcinogenicity. The majority of these studies assessed retrospective occupational cohorts and exposures within the chemical production industry [4–11]. Others include case-control studies investigating site-specific cancers in either occupational or community settings [12–17]. Three ecological studies examined the association between cancer incidence and residential proximity to sources of contamination where EDC was present [18–20]. Among these studies, excess risks of brain, pancreatic, lymphatic, hematopoietic, and stomach cancers have been reported. EDC may be associated with these excess risks, alone or in combination with other chemicals. Due to the lack of adequate exposure metrics for EDC, none of the studies are able to demonstrate a direct linkage of carcinogenic effects to EDC exposure alone [2,21].

EDC has been characterized as a probable human carcinogen (Group B2) by US EPA [22], a possible human carcinogen by IARC (Group 2B) [21] and is reasonably anticipated to be a human carcinogen by NTP (11th Report on Carcinogens) based mainly on evidence from animal studies. Target tissues identified in bioassays in mice and rats included lung, liver, mammary gland, bile duct and forestomach in both sexes, as well as uterine and testes of multiple rodent species by inhalation, gavage and dermal routes of exposure [23–27]. Further analysis of the mechanisms of these tumors in animal studies, particularly in specific target tissues, may inform the role of EDC and its metabolites in human carcinogenicity.

2. Metabolism of EDC

In evaluating the genotoxicity of EDC, it is important to consider the metabolic pathways and potential formation of genotoxic metabolites. EDC is rapidly and extensively absorbed through the lungs, gastrointestinal tract, and skin. Following absorption, EDC is distributed throughout all tissues of the body and is principally eliminated via biotransformation. A minor, yet significant fraction of the absorbed dose (typically less than 15%) is excreted as unchanged parent compound in exhaled air. Biotransformation of EDC is mediated through both an oxidative pathway, presumably via cytochrome P450-2E1 (CYP2E1), as well as through glutathione conjugation [28,29]. Metabolism of EDC occurs rapidly with a reported elimination half life of 20–30 min in male Osborne-Mendel rats following EDC inhalation and oral dosing, with the majority of elimination attributed to metabolism [30].

The proposed pathways for EDC metabolism are based on the metabolites that have been identified from *in vivo* and *in vitro* studies of EDC and ethylene dibromide (Fig. 1). CYP450 mediated mixed function oxidation of EDC results in chemically reactive 2-chloroacetaldehyde, a compound that may bind to cellular macromolecules (not shown) or undergo further biotransformation to form 2-chloroacetic acid or 2-chloroethanol [28,31]. 2-Chloroacetic acid can undergo glutathione conjugation to yield S-carboxymethyl-glutathione. S-carboxymethyl-glutathione can also be produced through glutathione conjugation of 2-chloroacetaldehyde and subsequent oxidation of the presumed intermediate S-(2-formylmethyl)-glutathione by aldehyde dehydrogenase [32]. S-carboxymethyl-glutathione is rapidly metabolized to yield S-carboxymethyl-cysteine, which is further oxidized to form thiodiacetic acid and thiodiacetic acid sulfoxide [27,30,31].

The direct conjugation of EDC with GSH is catalyzed by glutathione S-transferase(s) to yield S-(2-chloroethyl)-glutathione, a sulfur-half-mustard that has been demonstrated to be an alkylating agent [33]. This route of biotransformation potentially competes with conjugation of the oxidative pathway metabolite, 2-chloroacetaldehyde, as both conjugation steps consume GSH. Livesey and Anders [34] have demonstrated that S-(2-chloroethyl)-glutathione may be converted to ethylene in the presence of GSH. In addition, S-(2-chloroethyl)-glutathione may, via a reactive episulfonium ion intermediate, be converted to S,S′-ethylene-bis-(glutathione) or S-(2-hydroxyethyl)-glutathione in the presence of GSH or water, respectively [32,35]. S,S′-ethylene-bis-(glutathione) is presumed to be subject to degradation to S-S′-ethylene-bis-cysteine in the liver and kidney [31]. S-(2-hydroxyethyl)-glutathione may be oxidized to form S-(2-formylmethyl)-glutathione and subsequently biotransformed to S-carboxymethyl glutathione, as also occurs via the P450 biotransformation pathway [32]. Alternatively, S-(2-hydroxyethyl)-glutathione can be further processed to S-(2-hydroxyethyl)-cysteine and N-acetyl-S-(carboxymethyl)-cysteine [36,37].

The available animal data provide evidence that the majority of absorbed EDC is metabolized via oxidation mediated by CYP450s, with cytosolic glutathione conjugation representing a minor pathway [28]. For low EDC concentrations this is likely to be the case as CYP450 oxidation of EDC has been shown to exhibit high affinity, but low capacity metabolism [38,39]. Conversely, the GSH metabolic pathway of EDC displays low affinity but high capacity [38]. Therefore, as blood and tissue levels of EDC increase, oxidative metabolism likely becomes saturated, and GSH conjugation becomes the predominant metabolic pathway. This metabolic saturation through the CYP2E1 pathway appears to occur at EDC blood levels of 5–10 μg/ml, which corresponds to inhalation exposures of ∼150 ppm and oral doses of ∼25 mg/kg in rats [30,40]. Similar conclusions were made for trichloroethylene (TCE) based on animal studies, but a recent review and subsequent PBPK model-based analysis of the available *in vitro* and *in vivo* data [41,42] suggest that GSH conjugation may play a greater role in the bioactivation of TCE in humans than previously expected. Pharmacokinetic data from humans exposed at low concentrations are not available for EDC, nor has a PBPK model incorporating GSH metabolites been developed.

3. Genotoxicity of EDC

The application of genotoxicity data to predict potential carcinogenicity is based on the principle that genetic alterations are found in all cancers. Genotoxicity is the ability of chemicals to alter the genetic material in a manner that may lead to transmission during cell division. Risk evaluations of chemicals with regard to genotoxicity are generally based on a combination of tests that inform three major types of genetic damage: gene mutation (point mutations or deletions/insertions), clastogenicity (structural chromosomal aberrations) and aneuploidy (numerical chromosomal aberrations) [43]. Although most tests for mutagenicity detect changes in DNA or chromosomes, some specific modifications of the epigenome including proteins associated with DNA or RNA, can also cause transmissible changes.

Evaluation of genotoxicity data entails a weight of evidence approach that includes consideration of the various types of genetic damage that can occur. In acknowledging that genotoxicity test batteries are by design complementary evaluations that together detect the major mechanisms of genotoxicity, a recent International Programme on Chemical Safety (IPCS) publication [43] notes that "multiple negative results may not be sufficient to remove concern for mutagenicity raised by a clear positive result in a single mutagenicity assay". These considerations inform the