SUMMARY OF DATA FOR CHEMICAL SELECTION

Diisopropylcarbodiimide

CAS No. 693-13-0

Prepared for NCI by Technical Resources International, Inc

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CHEMICAL IDENTIFICATION

CAS Registry Number: 693-13-0

Chem. Abstr. Name: N,N'-Methanetetraylbis(2-propanamine)

Synonyms: DIC; diisopropylcarbodiimide; N,N'-diisopropylcarbodiimide; 1,3-diisopropylcarbodiimide

Structure, Molecular Formula and Molecular Weight:

$C_7H_{14}N_2$ Mol. wt.: 126.20

Chemical and Physical Properties:

From Aldrich Chemical Co., Inc. (1988) *Aldrich Technical Information Bulletin No. AL-168: 1,3-Diisopropylcarbodiimide (DIC)*, Milwaukee, WI.

Description: Colorless liquid

Boiling-point: 145-148°C

Solubility: Soluble in chloroform, methylene chloride, acetonitrile, dioxane, dimethylformamide, and tetrahydrofuran

Reactivity: Flammable and moisture-sensitive; reacts with water to form 1,3-diisopropylurea

Density: 0.806 g/ml

Refractive Index: $nD_{20} = 1.4330$

Flash Point: 33°C (Closed Cup)

Technical Products and Impurities: Diisopropylcarbodiimide is available in purities ranging from

97% to 99% and in quantities ranging from research quantities to 1000 kg lots. The main impurities are the unreacted isocyanates and polymerized carbodiimides (Kuney, 1990; Janssen Chimica, 1990).

BASIS OF NOMINATION TO THE CSWG

Diisopropylcarbodiimide (DIC) was nominated to the CSWG based on the following considerations:

DIC has been used in industry as a stabilizing agent, coupling agent, and condensing agent. The potential for exposure exists during the synthesis of polypeptides and other chemicals in the chemical and pharmaceutical industries, as well as during protein synthesis in the recombinant DNA industry. Two recent letters in *Chemical & Engineering News* highlight the hazards of accidental exposures to alkylcarbodiimides, which are highly toxic irritants, contact allergens, and skin sensitizers. Moyer (1990) described a delayed, temporary blindness due to damage to the outer layer of the cornea following an acute exposure to diisopropylcarbodiimide vapor. Ellis (1991) noted that the injury resembled mild to moderate mustard gas injuries and that, in view of the observed biological effects and chemistry, he believes that it is reasonable to assume that all alkylcarbodiimides are capable of functioning as alkylating agents and are therefore potential vesicants and carcinogens.

Evidence on which to evaluate the potential for human carcinogenicity is lacking. No epidemiological studies or case reports associating DIC with a cancer risk in humans have been reported. No information was found on the carcinogenicity in experimental animals, genotoxicity, teratogenicity, or metabolism of DIC. Experimental animal data is limited to an oral LD50 value of 36 mg/kg in mice.

INPUT FROM GOVERNMENT AGENCIES

The Interagency Testing Committee has provided the following information: DCC is a member of an ITC computerized substructure-based group. It has the potential to cause adverse effects to mammals.

SELECTION STATUS

ACTION BY CSWG: 9/26/91

Studies Requested: Nominated as a pair with dicyclohexylcarbodiimide for testing in general toxicity studies with further testing to be considered after results are known.

Priority: Moderate

Comments: Nominated as a pair with dicyclohexylcarbodiimide on the basis that these comnpounds show acute toxicity, that they're widely used and use in the field of bioenergetics is increasing, that there is a widespread low-level exposure, and that there is an absence of data on health effects. It was noted also that these two compounds are key chemicals in the carbodiimide class.

EXPOSURE INFORMATION

Commercial Availability

Production and Producers: Diisopropylcarbodiimide (DIC) is manufactured by extended

or excessive heating of isopropyl isocyanate from 100°C to 250°C under anhydrous conditions to condense the carbodiimide with elimination of carbon dioxide. A number of catalysts are effective in accelerating this reaction to the extent of making it a practical synthesis for this symmetrical carbodiimide. The phospholine oxides are particularly effective catalysts, although simple trialkyl-phosphine oxides or even triethyl phosphate may be used (Chadwick & Cleveland, 1979). Other organo-metallic catalysts, including tetraisopropyltitanate and tetraisopropylzirconate, are also used in industry to produce DIC (Smeltz, 1969; Budnick, 1968).

In addition, preparation of DIC has been proposed by the following two methods. N,N'-Diisopropylthiourea reacted with cyanuric chloride in dichloromethane yielded an oily product, which, when hydrolyzed with sodium hydroxide and heated, gave diisopropylcarbodiimide and trithiocyanuric acid (Furumoto, 1971b). Similarly, DIC can be obtained by treating N,N'-diisopropylthiourea in dichloromethane with dichlorodicyanobenzoquinone; the resultant mixture is evaporated and heated in sodium hydroxide to yield DIC (Furumoto, 1971a).

A process for producing DIC was patented by the Celanese Corp. in 1967 (White & Mullin, 1967). In this process, a reaction mass consisting of diisopropylthiourea, lead oxide and water is heated to the refluxing temperature, the mixture is distilled, and DIC is separated by decantation.

Fike Chemicals, Inc. is listed in TSCAPP (1991) as having manufactured DIC in 1977; the volume was reported as an unknown quantity. Artel Chemical Corp. was reported to the US International Trade Commission (1987) as a manufacturer of DIC in 1986, with no production level reported. No other industrial manufacturer was found.

Suppliers of DIC in research quantities include (Kuney, 1991; Directories Publishing Co., Inc., 1985; OPD Chemical Buyers Guide, 1991):

- Aldrich Chemical Co., Inc.
- Atomergic Chemicals Corp.
- Chemical Procurement Labs, Inc.
- Columbia Organic Chemical Co., Inc.
- Crescent Chemical Co., Inc.
- CTC Organics
- Davos Chemical Corp.
- Eastern Chemical, Division of United-Guardian, Inc.
- Fluka Chemical Corp.
- Janssen Chimica, Div. Janssen Pharmaceutica.
- K&K Laboratories, Div of ICN Biochemicals, Inc.
- Lachat Chemicals, Inc.
- Pfaltz & Bauer, Inc.
- Schweizerhall, Inc.
- Sigma Chemical Co.

Use Pattern: Diisopropylcarbodiimide can be used in most applications that use

dicyclohexylcarbodiimide (DCC). It is a very useful reagent for peptide syntheses, especially solid-phase peptide synthesis (SPPS). Although the coupling efficiency of DIC and DCC are virtually identical, DIC offers several advantages over DCC. The urea by-product of DIC, diisopropylurea, is easier to remove from the solid phase than the urea by-product of DCC, dicyclohexylurea, because it is more soluble in organic solvents. In addition, because DIC is a liquid, it is easier to dispense than DCC, which is crystalline with a low melting point (34-35°C) (Aldrich Chemical Co., Inc., 1988).

DIC is used as a peptide coupling reagent in the synthesis of: two protected peptide proteins of scorpion neurotoxin II; the N-hydroxysuccinamide active ester of diethylenetriaminepentaacetic acid (DTPA) which is subsequently used in a process for conjugating DTPA to proteins; N-acyl ureas; and 2-alkoxyoxazolones from alkoxycarbonylamino acids. It has also been used as a condensing reagent in dipeptide synthesis (Bates *et al*., 1981; Orlowska *et al*., 1983; Izdebski & Pelka, 1984; Kricheldorf *et al*., 1985; Paxton *et al*., 1985; Sabatier *et al*., 1987).

Hydrosilylation of DIC has also been used to synthesize N-silylformamides (Ojima *et al*., 1974).

In addition, DIC is used in the preparation of polyimide precursor coatings for electrophoretic image display fabrication (Minnema & Van der Zande, 1988).There are numerous other proposed uses for DIC. It has been reported that insoluble resin-bound DIC, in the presence of 1-hydroxybenzotriazole, catalyzed the synthesis of the cyclic peptide gramicidin S (Nutt, 1978). Also, alpha,beta-dehydroamino acid derivatives can be made from serine, threonine or cysteine using DIC (Miller, 1980).

DIC has been used as stabilization reagent for solution of S-(diisopropylaminoethyl)-O-ethyl methylphosphonothioate (Buckles & Lewis, 1977). In organic synthesis, DIC has been used in cycloaddition reactions to form a number of heterocyclic compounds (Aldrich Chemical Co., Inc., 1988). Like most carbodiimides, DIC has also been used in dehydration reactions for conjugated alkadienoic acid and anhydride preparations. In the presence of (dimethylamino)pyridinium toluenesulfonate as a catalyst, DIC has been used to prepare polyester from hydroxyphenyl-terminated carboxylic acids (Moore & Stupp, 1990).

DIC is used as a stabilizer for the military nerve agent, sarin (Nasr *et al*., 1988).

Human Exposure: There is potential exposure to DIC used as a stabilizer in sarin (a nerve agent that is part of the military chemical arsenal) and in handling the compound during the synthesis of peptides and other compounds.

Environmental Occurrence: No information on the occurrence or fate of DIC was found in the published literature [see Search Resource List].

Regulatory Status: No standards or guidelines have been set for occupational exposures to or environmental levels of DIC.

EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Human Data: No epidemiological studies or case reports associating DIC with a cancer risk in humans was found in the published literature [see Search Resource List].

Delayed temporary blindness has been described following an acute occupational exposure to diisopropylcarbodiimide vapor in a worker who cleaned up a 1 liter spill. Appropriate protective equipment worn included a respirator, a laboratory coat, and impervious gloves. Approximately 12 to 18 hours later, the worker experienced hazy vision followed by mild pain which maximized 34 hours after the exposure. Damage to the outer layer of the cornea resulted in blindness which was restored over a two-week period (Moyer, 1990). This unexpected ocular reaction resembled in type, duration, development, and recovery, descriptions of mild to moderate mustard gas injuries. The postulated mechanism of action for mustard gas toxicity is alkylation of nucleophilic functional groups of intracellular components occuring within minutes of exposure. The affected cells are unable to function and die, causing the symptoms. Presumably, all alkylcarbodiimides are potentially capable of functioning as alkylating agents and are, therefore, suspect vesicants and carcinogens (Ellis, 1991).

Animal Data: No animal carcinogenicity tests for DIC were found in the published literature [see Search Resource List].

The oral LD50 in mice is 36 mg/kg (RTECS, 1991b).

Short-Term Tests: No information was found on the genotoxicity of DIC in the published literature [see Search Resource List]. Several studies, however, reported negative genotoxicity of the nerve agent sarin stabilized with DIC. When Chinese hamster ovary cells were exposed to 1.4 x 10-3 M sarin with or without metabolic activation, SCEs were not significantly increased and cytotoxicity was not induced (Nasr *et al*., 1988). In isolated rat hepatocytes exposed to 2.4 x 10-3 M, unscheduled DNA repair synthesis was not induced (Klein *et al*., 1987).

Metabolism: No information was found on the absorption, distribution, metabolism, or excretion of DIC in the published literature [see Search Resource List].

Other Biological Activity: While the chemistry of DIC and DCC are virtually identical, interactions with biomolecules differ. Two cases in point involve ATPase. DCC readily inactivates *E. coli* BF1-ATPase at 0.05 mM while DIC shows almost no inactivation at this concentration (Satre *et al*., 1979). The Ca2+-ATPase of sarcoplasmic reticulum vesicles is readily inactivated by both DIC and DCC, but at much different rates (Murphy, 1981).

Structure/Activity Relationships: No information was found in HSDB, CCRIS, or DART on the carcinogenicity, mutagenicity, or teratogenicity of carbodiimides. Carbodiimides were nominated for toxicity testing to the NTP but testing was deferred pending response from the submitter for a specific carbodiimide of concern (National Toxicology Program, 1991). However, RTECS (1991) cites one study on the oral reproductive toxicity of carbodiimide in rats. A dose of 2450 mg/kg caused pre-implantation mortality; 1750 mg/kg caused paternal effects (testes, epididymis, sperm duct, prostate, seminal vesicle, Cowper's gland, and accessory glands); 208 mg/kg effected post-implantation mortality and the live birth index; 2600 mg/kg also affected newborns (live birth index, growth statistics, and delayed effects).

No information was found in RTECS, CCRIS, DART, or HSDB on the carcinogenicity, teratogenicity, or metabolism of other carbodiimide compounds including dicyclohexylcarbodiimide (CAS No. 538-75-0), bis(2,6-diethylphenyl)carbodiimide (CAS No. 2162-75-6), bis(3-chloro-o-tolyl)carbodiimide (CAS No. 961-63-7), or (3-dimethylaminopropyl)ethylcarbodiimide hydrochloride (CAS No. 25952-53-0).

Ellis (1991) speculated, however, that all alkylcarbodiimides are capable of functioning as alkylating agents and are, therefore, potential vesicants and carcinogens. He noted that the unexpected, delayed, temporary blindness following an acute occupational exposure to diisopropylcarbodiimide vapor described by Moyer (1990) resembled in type, duration, development, and recovery, descriptions of mild to moderate mustard gas injuries. The postulated mechanism of action for mustard gas toxicity is the alkylation of nucleophilic functional groups of intracellular components, occurring within minutes of exposure and leading to cellular dysfunction and even cell death.

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