Executive Summary

538-75-0
Dicyclohexylcarbodiimide

SUMMARY OF DATA FOR CHEMICAL SELECTION

CHEMICAL IDENTIFICATION

CAS Registry Number: 538-75-0

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

Synonyms and Trade Names: Bis(cyclohexyl)carbodiimide; carbodicyclohexylimide; DCC; DCCD; DCCI; dicyclohexylcarbodiimide; 1,3-dicyclohexylcarbodiimide; N,N'-dicyclohexylcarbodiimide

Structure, Molecular Formula and Molecular Weight:

$C_{13}H_{22}N_2$ Mol. wt.: 206.32

Chemical and Physical Properties:

Description: White crystals with a heavy sweet odor (Sax & Lewis, 1987)

Boiling-point: 98-100°C (0.5 mm Hg); 138-140°C (2 mm Hg); 154-156°C (11 mm Hg) (Sax & Lewis, 1987; Budavari, 1989)

Melting-point: 34-35°C (Budavari, 1989)

Solubility: Soluble in organic solvents (Sax & Lewis, 1987)

Reactivity: Moisture-sensitive (Kuney, 1990)

Flash Point: >110°C (Closed cup) (Aldrich Chemical Co., Inc., 1990)

Technical Products and Impurities: Dicyclohexylcarbodiimide is available in purities ranging from 99% to 99.5% in quantities ranging from research quantities to 1000 kg lots (Kuney, 1990; Van, 1990). The main impurities are the unreacted isocyanates and the polymerized carbodiimides (Chadwick & Cleveland, 1979).

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Various auxiliary agents have been used to suppress racemization; these include: N-hydroxybenzotriazole, N-hydroxysuccinamide, N-hydroxyphthalimide, endo-N-hydroxy-5-norbornene-2,3-dicarboximide, 3,4-dihydro-1,2,3-benzotriazin-4(3H)one, and copper(II) chloride (Lancaster Synthesis Inc., 1991).

UCARLNK Crosslinker XL-29 SE is a formulation produced by Union Carbide Chemicals and Plastics Co., Inc. and supplied as a 50% active solution in propylene glycol monomethyl ether acetate (Anon., 1990).

BASIS OF NOMINATION TO THE CSWG

Dicyclohexylcarbodiimide (DCC) was nominated to the CSWG based on the following considerations:

DCC has been used in industry since the early 1950s as a stabilizing agent, coupling agent, and condensing agent. The potential for exposure exists during its use in the synthesis of polypeptides in the chemical and pharmaceutical industries, as well as during protein synthesis in the recombinant DNA industry.

Carbodiimides are reported to be irritating to the skin, eyes, and respiratory tract. Occupational contact dermatitis due to DCC has been reported in laboratory workers since the late 1950s, and DCC vapor has been reported to cause acute ophthalmitis. 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

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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 DCC with a cancer risk in humans have been reported. No information was found on the carcinogenicity in experimental animals, genotoxicity, teratogenicity, or metabolism of DCC. However, DCC has been reported to inhibit protein translocating ATPases in biological membrane transport systems and enzyme complexes. Thus, a data gap exists for this biologically active, widely used reagent chemical.

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 and is listed in NIOSH's NOES.

SELECTION STATUS

ACTION BY CSWG: 9/26/91

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

Priority: Moderate

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

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EXPOSURE INFORMATION

Commercial Availability

Production and Producers: Dicyclohexylcarbodiimide (DCC) is manufactured by extended or excessive heating of cyclohexyl isocyanate 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 also be used. The dimer is not an intermediate in the uncatalyzed reaction; in the presence of a phosphine oxide catalyst, a four-membered-ring intermediate is highly probable (Chadwick & Cleveland, 1979).

In addition, preparation of DCC has been proposed by the following two methods. N,N'-Dicyclohexylthiourea reacted with cyanuric chloride in dichloromethane yields an oily product, which, when hydrolyzed with sodium hydroxide and heated, gives DCC and trithiocyanuric acid (Furumoto, 1971b). Similarly, DCC can be obtained by treating N,N'-dicyclohexylthiourea with dichlorodicyanobenzo-quinone in dichloromethane; the resultant mixture is evaporated and heated in sodium hydroxide to yield DCC (Furumoto, 1971a).

Suppliers of DCC in research quantities include (Kuney, 1990; Directories Publishing Co., Inc., 1985; Van, 1990):

- Aceto Corp.
- Accurate Chemical & Scientific Corp.
- Aldrich Chemical Co., Inc.
- Atomergic Chemicals Corp.
- Chemical Dynamic Corp.
- Chemical Procurement Labs, Inc.
- Chemtech Research Inc.
- Crystal Chemical Inc.

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- Chugai Boyeki (America) Corp.
- Davos Chemical Corp.
- Eastern Chemical, Division of United-Guardian, Inc.
- EM Industries., Inc., Fine Chemicals Div.
- EM Science, An EM Industries Co.
- Fluka Chemical Corp.
- ICN Biochemicals, Div. of ICN Biomedicals, Inc.
- Janssen Chimica, Div. Janssen Pharmaceutica
- Jonas Chemical Corp., Specialty Chemicals Div.
- K&K Laboratories, Div.of ICN Biochemicals, Inc.
- Lachat Chemicals, Inc.
- Lancaster Synthesis Inc.
- Mallinkrodt, Inc.
- Maypro Industries., Inc.
- Mitsui & Co. (U.S.A.) Inc.
- Pfaltz & Bauer, Inc.
- Pierce Chemical Co.
- Polysciences, Inc.
- Research Plus, Inc.
- ROC/RIC Inc.
- Schwarz/Mann Biotech, Div. of ICN Biomedicals, Inc.
- Schweizerhall, Inc.
- Sigma Chemical Co.
- Spectrum Chemical Mfg. Corp.
- Tanabe U.S.A., Inc., Chemical Sales Div.
- United States Biochemical Corp.

EM Laboratories imported between 1,000 and 10,000 pounds of DCC in 1977 (TSCAPP, 1991).

Use Pattern: The reactivity of DCC towards many functional groups has led to several

significant industrial and laboratory applications. Although carbodiimides were discovered in 1873, it was not until the early 1950s that they were used in industry. Their special reactivity with free carboxyl groups made them valuable as stabilizing agents in elastomers, natural rubber, and many types of polyolefins, polyesters, resins, fibers, cellulose esters, and foam materials to protect against deterioration. DCC has also been used as a coupling agent to produce natural lubricating oils by combining free carboxyl and amino groups in the oil to neutral amide groups (Azzi *et al.*, 1984).

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The application of DCC-induced formation of an amide bond was subsequently used for many other purposes, among which is the chemical synthesis of peptides. It has been used particularly in the synthesis of polypeptides with high molecular weights from low-molecular weight polypeptides. As the manufacture of polypeptides is a rapidly growing segment of the chemical and pharmaceutical industries, the use of DCC is expected to increase. This technique has also been applied in the preparation of different gels for affinity chromatography and in a method for the quantitative modification and estimation of carboxylic groups in proteins. In addition, DCC is increasingly used in the emerging industry of recombinant DNA as a coupling agent in protein synthesis (Azzi *et al.*, 1984; Hoffman & Adams, 1989).

In addition to being a widely used coupling agent for peptide bond formation, DCC is a coupling agent for the synthesis of amides, esters, and nucleotides. It has been used to synthesize: two protected peptide proteins of scorpion neurotoxin II; steroidal esters of carboxylic derivatives of N,N-bis(2-chloroethyl)aniline; and acylureas (Orlowska *et al.*, 1983; Pairas *et al.*, 1985; Sabatier *et al.*, 1987; Slebioda *et al.*,1990). It has also been used as a dehydrating reagent in the synthesis of soluble arylsilane aramids developed for use in high-performance epoxy clear-coat paints (Anon., 1991).

The reaction of carbodiimides with phosphoric esters and related phosphorus compounds has led to other uses in chemical synthesis. In 1953 it was discovered that carbodiimides are potent condensing agents for mono- and diesters of phosphoric acid and for the corresponding di- and tetraesters of pyrophosphoric acid. Since then, carbodiimides and DCC in particular have been widely employed in the synthesis of ortho- and pyrophosphate esters, nucleotides, cyclic phosphates,

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oligoribonucleotides, polynucleotides, nucleoside-5'-phosphoroamidates, and mixed anhydrides (Azzi *et al.*, 1984).

Carboxylic acids are converted by DCC to the anhydrides, while mono- and di-alkyl phosphates are converted to the corresponding pyrophosphates. Sulfinic acids can be converted to alkyl sulfinates and sulfinamides by DCC-mediated coupling with alcohols and amines, respectively. In combination with carbon disulfide, DCC converts arylamines to isothiocyanates (Lancaster Synthesis Inc., 1991).

DCC has been used as a reagent for peptide-condensing reactions, for coupling reactions between acidic species and nucleophiles, and for the synthesis of 1-beta-(isoxazolin-5-one-2-yl)alanine and N-substituted 3,4-unsubstituted isoxazolin-5-ones (Budavari, 1989; Kuney, 1990).

DCC can be used in various dehydration reactions. It dehydrates primary amides and aldoximes to nitriles, oximes to chiral oximes, and nitro-aldols to nitroalkenes (Lancaster Synthesis Inc., 1991).

DCC is one of a variety of reagents which, in combination with DMSO under the mild conditions, effect the oxidation of alcohols to carbonyl compounds, such as the conversion of cholan-24-ol to the corresponding aldehyde. DCC reacts with phenols to give O-aryl isoureas, which can be hydrogenolyzed to arenes over palladium on carbon in acetic acid. DCC also reacts exothermically with methanol in the presence of copper(I) chloride to give O-methyl isourea, which has found use in the N-methylation of amino-acids (Lancaster Synthesis Inc., 1991).

Hydrosilylation of DCC in the presence of catalytic amounts of $PdCl_2$ has also been used to synthesize N-silylformamidines, which are

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precursors to formamidines and acetylformamidines (Ojima *et al.*, 1974).

DCC also has several laboratory applications. A method for the gas chromatographic determination of trace amounts of sodium monofluoroacetate (a rodenticide) in aqueous solutions has been described that uses 0.8 mmol DCC and 1 mmol 2,4-dichloroaniline in the derivatization reaction (Ozawa & Tsukioka, 1987). A colorimetric method for the determination of pyridyl and pyrimidinyl compounds has been established in which DCC cleaves the ring to glutaconaldehyde or malonaldehyde which then react with DMBA to produce chromophores (Chen, 1985). DCC has also been used as a fluorogenic spray reagent for the detection of citric, isocitric, cis- and trans-aconitic acids, and some other biogenic compounds (Chen, 1982).

Since it was first discovered in 1967 that DCC inhibits proton-translocating ATPase in mitochondria, DCC has been widely used in the field of bioenergetics. Studies have used the inhibitory effect of DCC on proton translocating ATPases, Ca^{2+}-ATPases, and $(Na^{+}+K^{+})$ATPases to determine the structure and function of membrane transport systems and enzyme complexes. These have included proton ATPases in mitochondria, chloroplasts, bacteria, and chromaffin granules (the catecholamine vesicles of the adrenal medulla); Ca^{2+}-ATPase of sarcoplasmic reticulum; mitochondrial nicotinamide nucleotide transhydrogenase; cytochrome c oxidase (the terminal electron-transporting enzyme of the respiratory chain of mitochondria and bacteria); and the cytochrome b-c_1 complex of the mitochondrial respiratory chain (Azzi *et al.*, 1984). Recent studies have used DCC-induced inhibition of proton-translocating ATPases to investigate *S. cerevisiae* (Lee & Lee, 1989), rat heart mitochondria (Casey *et al.*, 1979), and rat liver mitochondria (Gauthier & Diwan, 1979). DCC has also been widely used to evaluate the mechanism of Na^{+}/H^{+} exchange in isolated rat renal brush border membrane vesicles (Kinsella **et al.**,

1985; Friedrich *et al.*, 1986, 1987; Sokol *et al.*, 1987) and rabbit renal microvillus membrane vesicles (Igarashi & Aronson, 1985, 1987); K^+/H^+ exchange in rat liver mitochondria (Garlid *et al.*, 1986); the contribution of H^+-ATPases to proton secretion along dog nephron segments (Tejedor *et al.*, 1987); and Ca^{2+} inhibition in rabbit sarcoplasmic reticulum vesicles prepared from back and hind leg white muscle (Argaman & Shoshan-Barmatz, 1988). It has also been used to study whether a proton pump is part of the mechanism responsible for secretin-dependent biliary secretion of bicarbonate ions in pigs (Grotmol *et al.*, 1987).

Human Exposure: No information on human exposure to DCC was found in the published literature [see Search Resource List]. NIOSH has reported exposure data to DCC in their 1983 NOES database.

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

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

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EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

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

Occupational contact dermatitis to DCC has been reported in research laboratory workers since the late 1950's. Zschunke & Folesky (1975) first observed three research laboratory workers with contact dermatitis in 1959 and seven cases of contact dermatitis in pharmaceutical workers in 1975. Subsequent case reports of skin sensitization have since appeared (Simpson, 1979; White & MacDonald, 1979; Davies, 1983; Funfstuck *et al.*, 1986; Lang & Hensel, 1987). Most recently Hoffman & Adams (1989) reported two cases of contact allergic dermatitis to DCC used in protein synthesis. A 33-year-old research chemist developed severe, blistering dermatitis of the hands, forearms, neck, and face after working with DCC while synthesizing a precursor of an experimental drug. Patch testing of DCC at 0.1% and 0.05% concentration in petrolatum elicited strong positive reactions. Similarly, a 29-year-old chemist who had been working for several months with DCC developed acute vesiculobullous dermatitis after contact with it in full concentration. Patch testing revealed strong positive reactions to 0.1% and 0.05% DCC in acetone.

In addition, DCC vapor has been reported to cause four cases of acute chemical ophthalmitis (Changji, 1983). Another alkylcarbodiimide, diisopropylcarbodiimide (DIC), was recently reported to have caused delayed, temporary blindness following an acute occupational exposure to DIC 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).

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

Short-Term Tests: No information was found on the genotoxicity of DIC in the published literature [see Search Resource List].

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

Other Biological Activity: Bioenergetic studies have relied on the time-dependent, irreversible inhibitory effect of DCC to study the mechanism of proton translocation in a variety of ATPases and in enzyme complexes of the respiratory chain. A recent study, however, found that in the mucosa of the pig intestine DCC inhibits proline-*beta*-naphthylamidase in a reversible and noncompetitive manner. The mode of the inhibition is noncompetitive with the substrates, indicating binding at a site other than its substrate binding site (Takahashi & Takahashi, 1990).

The metabolic effects of DCC in suspensions of dog nephron segments has been studied. DCC inhibited a ouabain-insensitive activity responsible for respiration in proximal tubules but not in the thick ascending limbs. However, measurement of cellular ATP, ADP, and AMP demonstrated that DCC interfered with phosphorylation but not respiration in aerobic tissues, probably at the functional oxygenase unit of mitochondrial H^+-ATPase. It was also found that the effect of DCC on ATP turnover in papillary connecting ducts may not reflect direct inhibition of a membrane proton pump (Tejedor *et al.*, 1987).

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It has also been reported that DCC elicited a sharp increase in the rate of oxygen consumption in guinea pig peritoneal neutrophils. The respiratory burst consisted of a lag phase during which NADPH oxidase underwent activation and a superoxide-producing phase (Aviram & Aviram, 1983).

Inactivation of hormone receptors in purified rat ovarian plasma membranes by DCC has been reported. Preincubation with 0.5 mM DCC reduced human chorionic gonadotropin binding by 50%; completely abolished hormone-sensitive adenylate cyclase (AC) activity stimulated by lutropin, follotropin, or human chorionic gonadotropin; but did not affect basal AC activity and activity stimulated by sodium fluoride or guanyl-5'-imidodiphosphate (Azulai & Salomon, 1979).

A Russian study (Leikina *et al.*, 1989) reported that DCC induced regular and specific changes of HeLa cell ultrastructure and that peculiarities of metabolism of the tumor cells as against normal cells caused weaker and slower response.

Structure/Activity Relationships: No information was found in HSDB, CCRIS, or DART on the carcinogenicity, mutagenicity, or teratogenicity of carbodiimides. The class of 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 effected newborns (live birth index, growth statistics, and delayed effects).

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No information was found in RTECS, CCRIS, DART, or HSDB on the carcinogenicity, teratogenicity, mutagenicity, or metabolism of other carbodiimide compounds including diisopropylcarbodiimide (CAS No. 693-13-0), 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, occuring within minutes of exposure and leading to cellular dysfunction and even cell death.

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Dicyclohexylcarbodiimide
538-75-0

NTP NOMINATION HISTORY AND REVIEW

A. Nomination History

1. Nomination Source: National Cancer Institute

2. Recommendations:
   - -General toxicity studies
   - -Consider for further testing after completion of toxicity studies

3. Rationale/Remarks:
   - -Nominated with diisopropyl-carbodiimide as a pair of key representative chemicals in the carbodiimde chemical class
   - -Widely used reagent in the chemical and pharmaceutical industries; increasing use in field of bioenergetics
   - -Widespread human exposure to low levels
   - -Evidence of acute toxicity
   - -Lack of adequate toxicity data
   - -Suspicion of carcinogenicity as a potential alkylating agent

4. Priority: Moderate

5. Date of Nomination: 6/93

B. Interagency Committee for Chemical Evaluation and Coordination Review

1. Date of Review:

2. Recommendations:

3. Priority:

4. NTP Chemical Selection Principles:

5. Rationale/Remarks:

SUMMARY OF DATA FOR CHEMICAL SELECTION

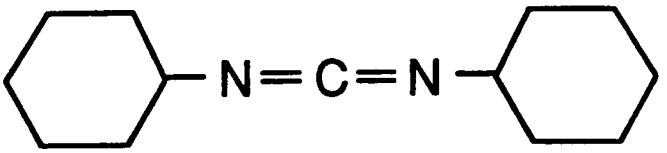
CHEMICAL IDENTIFICATION

CAS Registry Number: 538-75-0

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

Synonyms and Trade Names: Bis(cyclohexyl)carbodiimide; carbodicyclohexylimide; DCC; DCCD; DCCI; dicyclohexylcarbodiimide; 1,3-dicyclohexylcarbodiimide; N,N'-dicyclohexylcarbodiimide

Structure, Molecular Formula and Molecular Weight:



$C_{13}H_{22}N_2$ Mol. wt.: 206.32

Chemical and Physical Properties:

Description: White crystals with a heavy sweet odor (Sax & Lewis, 1987)

Boiling-point: 98-100°C (0.5 mm Hg); 138-140°C (2 mm Hg); 154-156°C (11 mm Hg) (Sax & Lewis, 1987; Budavari, 1989)

Melting-point: 34-35°C (Budavari, 1989)

Solubility: Soluble in organic solvents (Sax & Lewis, 1987)

Reactivity: Moisture-sensitive (Kuney, 1990)

Flash Point: >110°C (Closed cup) (Aldrich Chemical Co., Inc., 1990)

Technical Products and Impurities: Dicyclohexylcarbodiimide is available in purities ranging from 99% to 99.5% in quantities ranging from research quantities to 1000 kg lots (Kuney, 1990; Van, 1990). The main impurities are the unreacted isocyanates and the polymerized carbodiimides (Chadwick & Cleveland, 1979).

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Various auxiliary agents have been used to suppress racemization; these include: N-hydroxybenzotriazole,N-hydroxysuccinamide,N-hydroxyphthalimide,endo-N-hydroxy-5-norbornene-2,3-dicarboximide, 3,4-dihydro-1,2,3-benzotriazin-4(3H)one, and copper(II) chloride (Lancaster Synthesis Inc., 1991).

UCARLNK Crosslinker XL-29 SE is a formulation produced by Union Carbide Chemicals and Plastics Co., Inc. and supplied as a 50% active solution in propylene glycol monomethyl ether acetate (Anon., 1990).

BASIS OF NOMINATION TO THE CSWG

Dicyclohexylcarbodiimide (DCC) was nominated to the CSWG based on the following considerations:

DCC has been used in industry since the early 1950s as a stabilizing agent, coupling agent, and condensing agent. The potential for exposure exists during its use in the synthesis of polypeptides in the chemical and pharmaceutical industries, as well as during protein synthesis in the recombinant DNA industry.

Carbodiimides are reported to be irritating to the skin, eyes, and respiratory tract. Occupational contact dermatitis due to DCC has been reported in laboratory workers since the late 1950s, and DCC vapor has been reported to cause acute ophthalmitis. 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

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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 DCC with a cancer risk in humans have been reported. No information was found on the carcinogenicity in experimental animals, genotoxicity, teratogenicity, or metabolism of DCC. However, DCC has been reported to inhibit protein translocating ATPases in biological membrane transport systems and enzyme complexes. Thus, a data gap exists for this biologically active, widely used reagent chemical.

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 and is listed in NIOSH's NOES.

SELECTION STATUS

ACTION BY CSWG: 9/26/91

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

Priority: Moderate

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

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EXPOSURE INFORMATION

Commercial Availability

Production and Producers: Dicyclohexylcarbodiimide (DCC) is manufactured by extended or excessive heating of cyclohexyl isocyanate 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 also be used. The dimer is not an intermediate in the uncatalyzed reaction; in the presence of a phosphine oxide catalyst, a four-membered-ring intermediate is highly probable (Chadwick & Cleveland, 1979).

In addition, preparation of DCC has been proposed by the following two methods. N,N'-Dicyclohexylthiourea reacted with cyanuric chloride in dichloromethane yields an oily product, which, when hydrolyzed with sodium hydroxide and heated, gives DCC and trithiocyanuric acid (Furumoto, 1971b). Similarly, DCC can be obtained by treating N,N'-dicyclohexylthiourea with dichlorodicyanobenzo-quinone in dichloromethane; the resultant mixture is evaporated and heated in sodium hydroxide to yield DCC (Furumoto, 1971a).

Suppliers of DCC in research quantities include (Kuney, 1990; Directories Publishing Co., Inc., 1985; Van, 1990):

- Aceto Corp.
- Accurate Chemical & Scientific Corp.
- Aldrich Chemical Co., Inc.
- Atomergic Chemicals Corp.
- Chemical Dynamic Corp.
- Chemical Procurement Labs, Inc.
- Chemtech Research Inc.

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- Crystal Chemical Inc.
- Chugai Boyeki (America) Corp.
- Davos Chemical Corp.
- Eastern Chemical, Division of United-Guardian, Inc.
- EM Industries., Inc., Fine Chemicals Div.
- EM Science, An EM Industries Co.
- Fluka Chemical Corp.
- ICN Biochemicals, Div. of ICN Biomedicals, Inc.
- Janssen Chimica, Div. Janssen Pharmaceutica
- Jonas Chemical Corp., Specialty Chemicals Div.
- K&K Laboratories, Div.of ICN Biochemicals, Inc.
- Lachat Chemicals, Inc.
- Lancaster Synthesis Inc.
- Mallinkrodt, Inc.
- Maypro Industries., Inc.
- Mitsui & Co. (U.S.A.) Inc.
- Pfaltz & Bauer, Inc.
- Pierce Chemical Co.
- Polysciences, Inc.
- Research Plus, Inc.
- ROC/RIC Inc.
- Schwarz/Mann Biotech, Div. of ICN Biomedicals, Inc.
- Schweizerhall, Inc.
- Sigma Chemical Co.
- Spectrum Chemical Mfg. Corp.
- Tanabe U.S.A., Inc., Chemical Sales Div.
- United States Biochemical Corp.

EM Laboratories imported between 1,000 and 10,000 pounds of DCC in 1977 (TSCAPP, 1991).

Use Pattern: The reactivity of DCC towards many functional groups has led to several significant industrial and laboratory applications. Although carbodiimides were discovered in 1873, it was not until the early 1950s that they were used in industry. Their special reactivity with free carboxyl groups made them valuable as stabilizing agents in elastomers, natural rubber, and many types of polyolefins, polyesters, resins,

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fibers, cellulose esters, and foam materials to protect against deterioration. DCC has also been used as a coupling agent to produce natural lubricating oils by combining free carboxyl and amino groups in the oil to neutral amide groups (Azzi *et al.*, 1984).

The application of DCC-induced formation of an amide bond was subsequently used for many other purposes, among which is the chemical synthesis of peptides. It has been used particularly in the synthesis of polypeptides with high molecular weights from low-molecular weight polypeptides. As the manufacture of polypeptides is a rapidly growing segment of the chemical and pharmaceutical industries, the use of DCC is expected to increase. This technique has also been applied in the preparation of different gels for affinity chromatography and in a method for the quantitative modification and estimation of carboxylic groups in proteins. In addition, DCC is increasingly used in the emerging industry of recombinant DNA as a coupling agent in protein synthesis (Azzi *et al.*, 1984; Hoffman & Adams, 1989).

In addition to being a widely used coupling agent for peptide bond formation, DCC is a coupling agent for the synthesis of amides, esters, and nucleotides. It has been used to synthesize: two protected peptide proteins of scorpion neurotoxin II; steroidal esters of carboxylic derivatives of N,N-bis(2-chloroethyl)aniline; and acylureas (Orlowska *et al.*, 1983; Pairas *et al.*, 1985; Sabatier *et al.*, 1987; Slebioda *et al.*,1990). It has also been used as a dehydrating reagent in the synthesis of soluble arylsilane aramids developed for use in high-performance epoxy clear-coat paints (Anon., 1991).

The reaction of carbodiimides with phosphoric esters and related phosphorus compounds has led to other uses in chemical synthesis. In 1953 it was discovered that carbodiimides are potent condensing agents for mono- and diesters of phosphoric acid and for the

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corresponding di- and tetraesters of pyrophosphoric acid. Since then, carbodiimides and DCC in particular have been widely employed in the synthesis of ortho- and pyrophosphate esters, nucleotides, cyclic phosphates, oligoribonucleotides, polynucleotides, nucleoside-5'-phosphoroamidates, and mixed anhydrides (Azzi *et al.*, 1984).

Carboxylic acids are converted by DCC to the anhydrides, while mono- and di-alkyl phosphates are converted to the corresponding pyrophosphates. Sulfinic acids can be converted to alkyl sulfinates and sulfinamides by DCC-mediated coupling with alcohols and amines, respectively. In combination with carbon disulfide, DCC converts arylamines to isothiocyanates (Lancaster Synthesis Inc., 1991).

DCC has been used as a reagent for peptide-condensing reactions, for coupling reactions between acidic species and nucleophiles, and for the synthesis of 1-beta-(isoxazolin-5-one-2-yl)alanine and N-substituted 3,4-unsubstituted isoxazolin-5-ones (Budavari, 1989; Kuney, 1990).

DCC can be used in various dehydration reactions. It dehydrates primary amides and aldoximes to nitriles, oximes to chiral oximes, and nitro-aldols to nitroalkenes (Lancaster Synthesis Inc., 1991).

DCC is one of a variety of reagents which, in combination with DMSO under the mild conditions, effect the oxidation of alcohols to carbonyl compounds, such as the conversion of cholan-24-ol to the corresponding aldehyde. DCC reacts with phenols to give O-aryl isoureas, which can be hydrogenolyzed to arenes over palladium on carbon in acetic acid. DCC also reacts exothermically with methanol in the presence of copper(I) chloride to give

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O-methyl isourea, which has found use in the N-methylation of amino-acids (Lancaster Synthesis Inc., 1991).

Hydrosilylation of DCC in the presence of catalytic amounts of $PdCl_2$ has also been used to synthesize N-silylformamidines, which are precursors to formamidines and acetylformamidines (Ojima *et al.*, 1974).

DCC also has several laboratory applications. A method for the gas chromatographic determination of trace amounts of sodium monofluoroacetate (a rodenticide) in aqueous solutions has been described that uses 0.8 mmol DCC and 1 mmol 2,4-dichloroaniline in the derivatization reaction (Ozawa & Tsukioka, 1987). A colorimetric method for the determination of pyridyl and pyrimidinyl compounds has been established in which DCC cleaves the ring to glutaconaldehyde or malonaldehyde which then react with DMBA to produce chromophores (Chen, 1985). DCC has also been used as a fluorogenic spray reagent for the detection of citric, isocitric, cis- and trans-aconitic acids, and some other biogenic compounds (Chen, 1982).

Since it was first discovered in 1967 that DCC inhibits proton-translocating ATPase in mitochondria, DCC has been widely used in the field of bioenergetics. Studies have used the inhibitory effect of DCC on proton translocating ATPases, Ca^{2+}-ATPases, and $(Na^+ + K^+)$ATPases to determine the structure and function of membrane transport systems and enzyme complexes. These have included proton ATPases in mitochondria, chloroplasts, bacteria, and chromaffin granules (the catecholamine vesicles of the adrenal medulla); Ca^{2+}-ATPase of sarcoplasmic reticulum; mitochondrial nicotinamide nucleotide transhydrogenase; cytochrome c oxidase (the terminal electron-transporting enzyme of the respiratory chain of mitochondria and bacteria); and the cytochrome b-c_1 complex of the mitochondrial

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respiratory chain (Azzi *et al.*, 1984). Recent studies have used DCC-induced inhibition of proton-translocating ATPases to investigate *S. cerevisiae* (Lee & Lee, 1989), rat heart mitochondria (Casey *et al.*, 1979), and rat liver mitochondria (Gauthier & Diwan, 1979). DCC has also been widely used to evaluate the mechanism of Na^+/H^+ exchange in isolated rat renal brush border membrane vesicles (Kinsella et al., 1985; Friedrich *et al.*, 1986, 1987; Sokol *et al.*, 1987) and rabbit renal microvillus membrane vesicles (Igarashi & Aronson, 1985, 1987); K^+/H^+ exchange in rat liver mitochondria (Garlid *et al.*, 1986); the contribution of H^+-ATPases to proton secretion along dog nephron segments (Tejedor *et al.*, 1987); and Ca^{2+} inhibition in rabbit sarcoplasmic reticulum vesicles prepared from back and hind leg white muscle (Argaman & Shoshan-Barmatz, 1988). It has also been used to study whether a proton pump is part of the mechanism responsible for secretin-dependent biliary secretion of bicarbonate ions in pigs (Grotmol *et al.*, 1987).

Human Exposure: No information on human exposure to DCC was found in the published literature [see Search Resource List]. NIOSH has reported exposure data to DCC in their 1983 NOES database.

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

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

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EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

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

Occupational contact dermatitis to DCC has been reported in research laboratory workers since the late 1950's. Zschunke & Folesky (1975) first observed three research laboratory workers with contact dermatitis in 1959 and seven cases of contact dermatitis in pharmaceutical workers in 1975. Subsequent case reports of skin sensitization have since appeared (Simpson, 1979; White & MacDonald, 1979; Davies, 1983; Funfstuck *et al.*, 1986; Lang & Hensel, 1987). Most recently Hoffman & Adams (1989) reported two cases of contact allergic dermatitis to DCC used in protein synthesis. A 33-year-old research chemist developed severe, blistering dermatitis of the hands, forearms, neck, and face after working with DCC while synthesizing a precursor of an experimental drug. Patch testing of DCC at 0.1% and 0.05% concentration in petrolatum elicited strong positive reactions. Similarly, a 29-year-old chemist who had been working for several months with DCC developed acute vesiculobullous dermatitis after contact with it in full concentration. Patch testing revealed strong positive reactions to 0.1% and 0.05% DCC in acetone.

In addition, DCC vapor has been reported to cause four cases of acute chemical ophthalmitis (Changji, 1983). Another alkylcarbodiimide, diisopropylcarbodiimide (DIC), was recently reported to have caused delayed, temporary blindness following an acute occupational exposure to DIC 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).

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

Short-Term Tests: No information was found on the genotoxicity of DIC in the published literature [see Search Resource List].

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

Other Biological Activity: Bioenergetic studies have relied on the time-dependent, irreversible inhibitory effect of DCC to study the mechanism of proton translocation in a variety of ATPases and in enzyme complexes of the respiratory chain. A recent study, however, found that in the mucosa of the pig intestine DCC inhibits proline-*beta*-naphthylamidase in a reversible and noncompetitive manner. The mode of the inhibition is noncompetitive with the substrates, indicating binding at a site other than its substrate binding site (Takahashi & Takahashi, 1990).

The metabolic effects of DCC in suspensions of dog nephron segments has been studied. DCC inhibited a ouabain-insensitive activity responsible for respiration in proximal tubules but not in the thick ascending limbs. However, measurement of cellular ATP, ADP, and AMP demonstrated that DCC interfered with phosphorylation but not respiration in aerobic tissues, probably at the functional oxygenase unit of mitochondrial H^+-ATPase. It was also found that the effect of DCC on ATP turnover in papillary connecting ducts may not reflect direct inhibition of a membrane proton pump (Tejedor *et al.*, 1987).

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It has also been reported that DCC elicited a sharp increase in the rate of oxygen consumption in guinea pig peritoneal neutrophils. The respiratory burst consisted of a lag phase during which NADPH oxidase underwent activation and a superoxide-producing phase (Aviram & Aviram, 1983).

Inactivation of hormone receptors in purified rat ovarian plasma membranes by DCC has been reported. Preincubation with 0.5 mM DCC reduced human chorionic gonadotropin binding by 50%; completely abolished hormone-sensitive adenylate cyclase (AC) activity stimulated by lutropin, follotropin, or human chorionic gonadotropin; but did not affect basal AC activity and activity stimulated by sodium fluoride or guanyl-5'-imidodiphosphate (Azulai & Salomon, 1979).

A Russian study (Leikina *et al.*, 1989) reported that DCC induced regular and specific changes of HeLa cell ultrastructure and that peculiarities of metabolism of the tumor cells as against normal cells caused weaker and slower response.

Structure/Activity Relationships: No information was found in HSDB, CCRIS, or DART on the carcinogenicity, mutagenicity, or teratogenicity of carbodiimides. The class of 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 effected newborns (live birth index, growth statistics, and delayed effects).

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No information was found in RTECS, CCRIS, DART, or HSDB on the carcinogenicity, teratogenicity, mutagenicity, or metabolism of other carbodiimide compounds including diisopropylcarbodiimide (CAS No. 693-13-0), 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, occuring within minutes of exposure and leading to cellular dysfunction and even cell death.

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