Screening Assessment for the Challenge

Ethanedial

Chemical Abstracts Service Registry Number
107-22-2

Environment Canada
Health Canada

September 2011

Synopsis

The Ministers of the Environment and of Health have conducted an assessment of ethanedial, Chemical Abstracts Service Registry Number 107-22-2[1]. The substance ethanedial was identified following the categorization of the *Domestic Substance List* as a high priority for action under the Challenge initiative under the Chemicals Mananagement Plan. Ethanedial was identified as a high priority as it was considered to pose intermediate potential for exposure (IPE) of individuals in Canada and is classified by other agencies on the basis of genotoxicity. This substance did not meet the ecological categorization criteria for persistence, bioaccumulation or inherent toxicity to aquatic organisms.

Hydrated forms of ethanedial can occur naturally. It is used in Canada in corrosion inhibitors and anti-scaling agents; as a finishing agent in textiles, paper and leather; as an intermediate in reactions to produce other substances for commercial use; as a processing aid for petroleum production; as a viscosity adjustor; as a paint and coating additive; and in pest control products. According to information reported under section 71 of CEPA 1999, Canadian companies imported over 136 000 kg in 2006. In addition, between 100 000 and 1 000 000 kg of ethanedial was used in Canada in that year (Canada 2009b). Between 10 000 and 100 000 kg of ethanedial was reported to be released into the environment in 2006, with the highest release to wastewater.

Based on available information on concentrations of ethanedial in the environment (water, soil and air) and food, as well as data submitted under section 71 of CEPA 1999, the general population is expected to be exposed to ethanedial primarily from environmental media (ambient, indoor air) and from its naturally-occuring presence in food. Additionally, the general population may be exposed to low levels of ethanedial resulting from its presence as a residual in certain consumer products, such as paint and face wash and from its use as a finishing agent in paper.

As ethanedial was classified on the basis of genotoxicity by the European Union, genotoxicity was a key focus for this screening assessment. Ethanedial tested positive in a range of in vitro assays for mutagenicity and genotoxicity. However, the results of in vivo tests indicated that genotoxicity occurred predominantly at the site of entry and in the liver, but not in distant tissues, when administered orally. Carcingenicity was not observed when ethanedial was administered dermally to mice for their lifespan (Cancer bioassays by the oral and inhalation routes have not been conducted). Based on the existence of protective mechanisms, it is expected that intracellular ethanedial concentrations must overcome a threshold before genotoxicity occurs. Therefore, a threshold approach is used to characterize risk to human health.

Non-cancer effects were observed in repeat-dose studies. Decreased body and organ weights, and decreased food intake, were the most consistently observed effects in rats

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exposed by the oral route in repeat-dose studies. Exposure, by inhalation, to ethanedial aerosols induced minimal squamous metaplasia of the rat epiglottal epithelium, while acute exposures to ethanedial vapour saturated atmospheres caused an increased breathing rate in rats. Repeated dermal exposures resulted in irritation and necrotic areas on the skin of some exposed mice. The margins between upper-bounding estimates of exposure and the critical effect levels are considered to be adequate to address uncertainties in health effects and exposure datebases. It is concluded that ethanedial is not a substance that is entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

Based on its physical and chemical properties, it is expected that if released to air, the substance partitions to soil and water, and that if released to soil or water, the substance will mostly remain in these compartments. Based on these considerations and the use pattern of ethanedial, the substance will mainly be found in water.

Based on empirical biodegradation studies, ethanedial is not expected to be persistent in the environment. It is also expected to have very low bioaccumulation potential based on modelled data. Ethanedial therefore does not meet the persistence or bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations*. It was also found to have low acute toxicity to aquatic organisms.

For the evaluation of ecological risk, conservative exposure scenarios were examined in which the six largest users/importers of ethanedial in Canada discharge ethanedial into the aquatic environment. The predicted environmental concentrations in water (PECs) at these sites were all below the predicted no-effect concentrations (PNEC) calculated for algae, which was the most sensitive type of aquatic organism.

Based on the information available, it is concluded that ethanedial is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

Based on the information available, it is concluded that ethanedial does not meet any of the criteria set out in section 64 of CEPA *1999*.

This substance will be considered for inclusion in the *Domestic Substances List* inventory update initiative. In addition and where relevant, research and monitoring will support verification of assumptions used during the screening assessment.

Introduction

The *Canadian Environmental Protection Act, 1999* (CEPA 1999) (Canada 1999) requires the Minister of the Environment and the Minister of Health to conduct screening assessments of substances that have met the categorization criteria set out in the Act to determine whether these substances present or may present a risk to the environment or to human health.

Based on the information obtained through the categorization process, the Ministers identified a number of substances as high priorities for action. These include substances that

- met all of the ecological categorization criteria, including persistence (P), bioaccumulation potential (B) and inherent toxicity to aquatic organisms (iT), and were believed to be in commerce in Canada; and/or
- met the categorization criteria for greatest potential for exposure (GPE) or presented an intermediate potential for exposure (IPE) and had been identified as posing a high hazard to human health based on classifications by other national or international agencies for carcinogenicity, genotoxicity, developmental toxicity or reproductive toxicity.

The Ministers therefore published a notice of intent in the *Canada Gazette*, Part I, on December 9, 2006 (Canada 2006a), which challenged industry and other interested stakeholders to submit, within specified timelines, specific information that may be used to inform risk assessment, and to develop and benchmark best practices for the risk management and product stewardship of those substances identified as high priorities.

The substance ethanedial was identified as a high priority for assessment of human health risk because it was considered to present IPE and had been classified by other agencies on the basis of genotoxicity (EU 1996). The Challenge for this substance was published in the *Canada Gazette* on September 26, 2009 (Canada 2009a, b). A substance profile was released at the same time. The substance profile presented the technical information available prior to December 2005 that formed the basis for categorization of this substance. As a result of the Challenge, submissions of information pertaining to the substance were received (Canada 2009b).

Although ethanedial was determined to be a high priority for assessment with respect to human health, it did not meet the categorization criteria for persistence, bioaccumulation potential or inherent toxicity to aquatic organisms.

Screening assessments focus on information critical to determining whether a substance meets the criteria set out in section 64 of CEPA 1999[2]. Screening assessments examine

[2] A determination of whether one or more of the criteria of section 64 are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposures in the general

scientific information and develop conclusions by incorporating a weight of evidence approach and precaution.

This final screening assessment includes consideration of information on chemical properties, hazards, uses and exposure, including the additional information submitted under the Challenge. Data relevant to the screening assessment of this substance were identified in original literature, review and assessment documents and stakeholder research reports and from recent literature searches, up to May 2010. Key studies were critically evaluated; modelling results may have been used to reach conclusions.

Evaluation of risk to human health involves consideration of data relevant to estimation of (non-occupational) exposure of the general population, as well as information on health hazards (based principally on the weight of evidence assessments of other agencies that were used for prioritization of the substance). Decisions for human health are based on the nature of the critical effect and/or margins between conservative effect levels and estimates of exposure, taking into account confidence in the completeness of the identified databases on both exposure and effects, within a screening context. The final screening assessment does not represent an exhaustive or critical review of all available data. Rather, it presents a summary of the critical information upon which the proposed conclusion is based.

This final screening assessment was prepared by staff in the Existing Substances Programs at Health Canada and Environment Canada and incorporates input from other programs within these departments. This assessment has undergone external written peer review/consultation. Comments on the technical portions relevant to human health were received from scientific experts selected and directed by Toxicology Excellence for Risk Assessment (*TERA*) and included comments by Dr. Bernard Gadgbui (*TERA*), Dr. Pam Williams (*E Risk* Sciences) and Dr. Susan Griffin (*U.S. Environmental Protection* Agency). The ecological portion of this assessment has undergone external written peer review/consultation. Additionally, the draft of this screening assessment was subject to a 60-day public comment period. While external comments were taken into consideration, the final content and outcome of the screening risk assessment remain the responsibility of Health Canada and Environment Canada.

The critical information and considerations upon which the assessment is based are summarized below.

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environment. For humans, this includes, but is not limited to, exposures from ambient and indoor air, drinking water, foodstuffs, and the use of consumer products. A conclusion under CEPA 1999 on the substances in the Chemicals Management Plan (CMP) Challenge Batches 1-12 is not relevant to, nor does it preclude, an assessment against the hazard criteria specified in the *Controlled Products Regulations*, which is part of regulatory framework for the Workplace Hazardous Materials Information System [WHMIS] for products intended for workplace use.

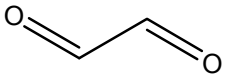
Substance Identity

Substance name

For the purposes of this document, this substance will be referred to as ethanedial, the DSL name for this substance.

Table 1. Substance identity for ethanedial

Chemical Abstracts Service Registry Number (CAS RN)	**107-22-2**
DSL name	**Ethanedial**
National Chemical Inventories (NCI) names[1]	*Ethanedial (TSCA, DSL, AICS, SWISS, PICCS, ASIA-PAC, NZIoC)* *Glyoxal (DSL, EINECS, ENCS, SWISS, PICCS)* *Oxalaldehyde (ECL)*
Other names	*1,2-Ethanedione* *Aurarez 136* *Biformal* *Biformyl* *Cartabond GHF* *Daicel GY 60* *Diformyl* *Ethanedione* *Glyfix CS 50* *Ethanedial aldehyde* *Glyoxal T 40* *Glyoxazal* *Glyoxazal GX* *Glyoxylaldehyde* *Gohsezal P* *GX* *GX (aldehyde)* *Oxal* *Permafresh 114* *Protorez BLF-C*
Chemical group (DSL Stream)	Discrete organics
Major chemical class or use	Aldehydes
Major chemical sub-class	Dialdehyde
Chemical formula	$C_2H_2O_2$

Chemical structure	
SMILES[2]	O=CC=O (anhydrous), OC(O)C(O)O (hydrated monomer)
Molecular mass	58.04 g/mol (anhydrous) , 94.07 g/mol (hydrated monomer)

[1] National Chemical Inventories (NCI). 2006: AICS (Australian Inventory of Chemical Substances); ASIA-PAC (Asia-Pacific Substances Lists); ECL (Korean Existing Chemicals List); EINECS (European Inventory of Existing Commercial Chemical Substances); ENCS (Japanese Existing and New Chemical Substances); NZIoC (New Zealand Inventory of Chemicals); PICCS (Philippine Inventory of Chemicals and Chemical Substances); and TSCA (Toxic Substances Control Act Chemical Substance Inventory; SWISS-(Switzerland Inventory of Notified New Substances)).

[2] Simplified Molecular Input Line Entry System

Pure, anhydrous ethanedial (structure and formula shown above) can only be produced in the laboratory and does not exist in a stable form (OECD 2003). Ethanedial is commonly supplied in the form of aqueous solution at 40% (w/w) (expressed as CHOCHO) (OECD 2003). In dilute aqueous solution, the hydrated monomer (ethane bis-gemdiol), shown in Figure 1, is the main form of ethanedial (OECD 2003). However, at higher concentrations, this monomer tends to polymerise to acetals-semiacetals, the presence of which depends on both the pH and the concentration of the solution. The main oligomeric forms are the dioxolane dimer (Figure 2) and the bis(dioxolane) trimer (Figure 3). In the environment, at lower concentrations (less than 1 *M* or 58 g/L), it can be assumed that only the monomer is present (OECD 2003, Whipple 1970). In a 40% solution, the monomer content is approximately 11%, the dimer and trimer forms being dominant (OECD 2003). According to Whipple (1970), the dimer form is the predominant species in ethanedial solutions between 1 and 10 *M*. Given that the commercially available 40% aqueous solution contains 8.75 *M* ethanedial (expressed as CHOCHO), the dominant ethanedial species in this solution would be the dioxolane dimer (Figure 2).

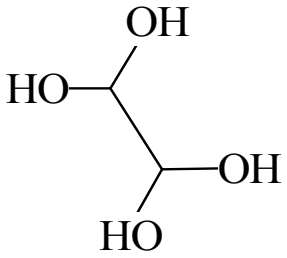


Figure 1. Hydrated ethanedial monomer

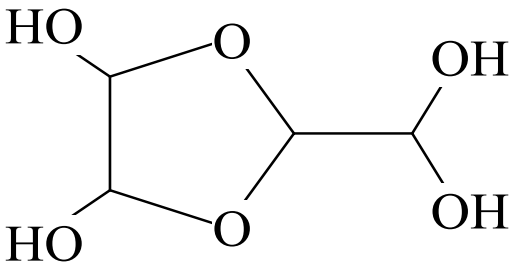


Figure 2. Hydrated ethanedial dimer

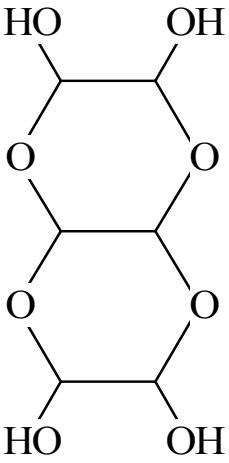


Figure 3. Hydrated ethanedial trimer

Physical and Chemical Properties

As discussed in Substance Identity section, ethanedial in pure form is not stable in the environment and will react to form the hydrated monomer. At environmentally relevant concentrations (ie. those associated with typical releases to the environment), the hydrated monomer form is the only form of ethanedial that will be present, and as such, this is the form that was used for modelling of physical and chemical properties and other properties, such as environmental fate, persistence and bioaccumulation.

Table 2 contains experimental and modelled physical and chemical properties of the hydrated monomer of ethanedial that are relevant to its environmental fate.

Table 2. Physical and chemical properties for ethanedial (hydrated monomer)

Property	Type	Value[1]	Temperature (°C)	Reference
Melting point (ºC)	Modelled	43		MPBPWIN 2008
Boiling point (ºC)	Modelled	252		MPBPWIN 2008
Density (kg/m^3)	-	1270[2]	-	-
Vapour pressure (Pa)	Modelled	0.10	25	MPBPWIN 2008
Henry's Law constant ($Pa \cdot m^3/mol$)	Experimental	2.4×10^{-4}* (4.19×10^5 M/atm)	25, pH 7	Ip et al. (2009)
		2.8×10^{-4} (3.6×10^5 M/atm)	25	Zhou and Mopper (1990)
		$\leq 3.38 \times 10^{-4}$ ($\geq 3.00 \times 10^5$ M/atm)	15-45	Betterton and Hoffmann (1988)
	Modelled, bond estimate	1.77×10^{-5}		HENRYWIN (2008)
Log K_{ow} (Octanol-water partition coefficient) (dimensionless)	Modelled	-1.3	25	KOWWIN 2008

Property	Type	Value[1]	Temperature (°C)	Reference
Log K_{oc} (Organic carbon-water partition coefficient) (dimensionless)	Modelled, (Molecular Connectivity Index method)	0.00		KOCWIN 2008
	Modelled, (based on log K_{OW})	0.24		KOCWIN 2008
Water solubility (mg/L)	Modelled	1×10^6	25	WSKOW, WATERNT 2008
pK_a (Acid dissociation constant) (dimensionless)	Modelled	Non-ionizing	-	ACD/pK_aDB (2005)

Abbreviations: K_{oc}, organic carbon-water partition coefficient; K_{ow}, octanol–water partition coefficient.
[1] Values in parentheses represent the original ones as reported by the authors or as estimated by the models.
[2] This value is for the 40% aqueous solution (Hoechst AG 1990a; Rieser 2008).
*indicates selected value for EQC modelling. Ip et al. (2009) was deemed to be the most robust of the three Henry's Law constant (HLC) studies, as this study measured the HLC at four temperatures in the range 278 to 318 K, the pH was measured, and the standard deviation of each measurement was reported. Also, at least five measurements were made at each temperature, while the value from Zhou et al. (1990) was based on only two measurements.

Sources

There are several natural sources of hydrated ethanedial. Ethanedial may be formed from humic acids by photochemical reactions in seawater or be released into the environment by natural fires along with other aldehydes (CICAD 2004, McDonald 2000). In addition, ozone can catalyze the formation of ethanedial from organic carbon, or aromatic compounds under abiotic conditions (CICAD 2004). Ethanedial is also endogenously produced during normal cellular metabolic processes, such as sugar autoxidation, and by oxidative stress, UV-photo damage, DNA oxidation and lipid peroxidation (CICAD 2004).

Ethanedial is also commonly detected in fermented and browned foods, such as wine, soya sauce, honey and bread crusts (da Silva Ferreira 2007, SCCP 2005, CICAD 2004, Weigel 2004). Heat processing reactions such as caramelization, roasting, frying or baking and the Maillard reaction of saccharides with proteins involving glucose contribute to the formation of ethanedial (Arribas-Lorenzo 2010, CICAD 2004).

The presence of ethanedial in both whole tobacco and mainstream cigarette smoke has been known since the early 1980s (Rodgman and Perfetti 2009, Moree-Testa and Saint-

Jalm 1981). However, this compound in mainstream cigarette smoke is extremely difficult to analyze because it is highly volatile, reactive and water soluble.

Ethanedial may be emitted into the environment from biomass burning or anthropogenic activities as a short-lived intermediate product in the oxidation of non-methane volatile organic compounds (Stavrakou et al. 2009). Ethanedial can be prepared by oxidation of acetaldehyde and selenious acid; commercially it is prepared by the gas phase oxidation of ethylene glycol in the presence of a silver or copper catalyst or by oxidation of acetaldehyde and nitric acid (CICAD 2004).

According to information reported under section 71 of CEPA 1999, Canadian companies imported over 136 000 kg in 2006. In addition, between 100 000 and 1 000 000 kg of ethanedial was used in Canada in that year.

Uses

Based on information identified in the scientific and technical literature, ethanedial is primarily marketed as a 40% aqueous solution, which is used as a starting material for a number of other compounds (OECD 2003, SCCP 2005, Rieser 2008). Given the dual functionality and ability for ethanedial to form heterocyclic compounds, ethanedial is commonly used in the production of resins and for cross-linking functionalized polymers, such as textiles, paper and proteins (OECD 2003, SCCP 2005, CICAD 2004). The application of ethanedial as a cross-linking agent in textiles, polishes, tanning processes and paper benefits these industries by leading to softer, less wrinkled fabrics, preserving leather quality, improving paper coating efficiency and increasing paper wet and dry strength (Rieser 2008, Thorn & Au 2009). The solvent ethanedial 40% is commonly used as an intermediate for paper and textile products and may be present in products as a residual. Ethanedial is also commonly used as an intermediate in the production of pharmaceuticals and dyestuffs (CICAD 2004). It is used for its reducing properties in the photographic industry and in glassmaking in the production of mirrors (OECD 2003, BG Chemie 1998).

Ethanedial is found in many products such as insecticides, cleaners and personal care products. Ethanedial is used as a biocidal active ingredient for disinfectants (Rieser 2008, SCCP 2005, CICAD 2004, OECD 2003). Most disinfectants containing ethanedial used in Canada are used in occupational settings in the health industry (e.g., hospitals); however, it may also be used in household cleaners in other countries (Rieser 2008, CICAD 2004).

Ethanedial is not currently listed on Health Canada's Cosmetic Ingredient Hotlist, which prohibits or restricts substances from being intentionally used as ingredients in cosmetics. According to the Cosmetic Notification System (CNS) in Canada, ethanedial is present as a residual in personal care products such as skin cleanser, skin moisturizer, manicure preparation and hair grooming (CNS 2010). The Cosmetic Directive in the European Union also reported the use of ethanedial as a starting material for ingredients used in personal care products, similar to those available in Canada (SCCP 2005). Concentrations

in personal care products would be expected to be low as ethanedial is present only as a residual from polymerising reactions in finished products.

According to submissions made under section 71 of CEPA 1999, industrial use patterns in Canada include corrosion inhibitors and antiscaling agents; finishing agents to paper and leather; intermediates consumed in a reaction to produce other substances for commercial use; processing aids for petroleum production; viscosity adjustors; residual in paint and coating additives/thickeners; pest control substances and residuals in paper cartons (Environment Canada 2010a).

Ethanedial is not listed in the Drug Product Database (DPD), the Therapeutic Products Directorate's Internal Non-Medicinal Ingredients Database nor the Natural Health Products Ingredients Database (NHPID) as a medicinal or non-medicinal ingredient in pharmaceutical drugs for human use, natural health products or veterinary products (DPD 2010, 2010 personal communication from Therapeutic Products Directorate, Health Canada; unreferenced, NHPID 2010)). However, as ethanedial is commonly used as a chemical intermediate in the synthesis of some pharmaceutical products, it may be present in trace amounts in certain pharmaceutical products. The Licensed Natural Health Products Database (LNHPD) lists ethanedial as a non-medicinal ingredient in one currently licensed natural health product, an acne facial cleanser (LNHPD 2010). Furthermore, the DPD lists ethanedial as an active ingredient at concentrations of 4% and 0.04% in two liquid disinfectant products that are used for cleaning medical instruments or in hospital facilities and not for general consumer use (DPD 2010).

The Pest Management Regulatory Agency (PMRA) lists ethanedial as an intentional ingredient in formulated pesticides with concentrations ranging from 3.3×10^{-5} – 0.07% w/w (April 2010 email from PMRA, Health Canada, to Risk Management Bureau, Health Canada; unreferenced).

Ethanedial may be naturally present in some foods; however, it is not listed as an approved food additive under the *Food and Drug Regulations* (Canada 2010). Ethanedial has not been identified to be present or used in formulations of incidental additives. For food packaging applications, ethanedial is a crosslinker for starch based materials. Ethanedial has also been indentified as a component of a processing aid used in the manufacture of paper or paperboard (2010 personal communication from Food Directorate - Food Packaging & Incidental Additives Section, Health Canada; unreferenced).

Releases to the Environment

Information reported under section 71 of CEPA 1999 indicated that less than 30 000 kg of ethanedial was released into the environment in 2006. The majority of those releases were to water and land, with small fractions released to air (Canada 2009b). In addition to environmental releases, <1000 kg of ethanedial was transferred to both hazardous and non-hazardous waste facilities (Canada 2009b).

Ethandial is not reported to the Canadian National Pollutant Release Inventory (NPRI) (NPRI 2006-2009), or to the U.S. Toxics Release Inventory (TRI) (TRI 2006-2009).

Environmental Fate

Level III equilibrium criterion fugacity (EQC) modelling was done for the hydrated ethanedial monomer (Table 3), the form most likely to be found in the environment. Based on the physical and chemical properties (Table 2), the results suggest that ethanedial in hydrated monomer form is expected to reside almost exclusively in water, if released to water, and will reside predominantly in soil, if released to soil or air, with most of the remainder being transported to water. Due to the very short half-life of ethanedial in air, little ethanedial remains in air at steady state. Ethanedial's negligible Henry's Law Constant of 2.4×10^{-4} Pa m^3/mol indicates that ethanedial is essentially non-volatile from the aqueous phase.

Based on the use pattern of ethanedial and the EQC modelling, the substance will mainly be found in water and soil with little partitioning to other compartments.

Table 3. Results of the Level III fugacity modelling for hydrated ethanedial monomer (EQC 2003)

	Percentage of substance partitioning into each compartment			
Substance released to:	**Air**	**Water**	**Soil**	**Sediment**
Air (100%)	0.014	22.7	77.2	0.034
Water (100%)	<0.01	99.9	<0.01	0.15
Soil (100%)	<0.01	15.8	84.2	0.023

Persistence and Bioaccumulation Potential

Environmental Persistence

The major tropospheric transformation processes for the α-dicarbonyls such as ethanedial are photolysis and reaction with the OH radical (Atkinson 2000). Atkinson (2000) calculated a lifetime of 1.1 days for ethanedial in the presence of hydroxyl radicals (assuming an average 12-hour daytime concentration of 2×10^6 molecules OH/cm^3) and a photolysis lifetime of 5 hours (for overhead sun). However, Atkinson (2000) notes that the photodissociation data for ethanedial have significant uncertainties.

Table 4a presents the empirical biodegradation data for ethanedial, 40% aqueous solution. In the 40% aqueous form, the hydrated dimer form predominates (see Figure 2). These data show considerable biodegradation over 7 to 28 days in various biodegradation tests, demonstrating that ethanedial is readily biodegradable. These tests show that the ultimate biodegradation half-life of ethanedial in water is shorter than 90 days, and, therefore, ethanedial (40% aqueous solution) is not considered to be persistent in water.

Table 4a. Empirical biodegradation data for 40% aqueous ethanedial

Method	Degradation value	Degradation endpoint, units	Test Duration (days)	Reference
OECD (1992a), Method 301C	65	%BOD	14	NITE (2002)[1]
OECD (1992a), Method 301D	90	% ThOD	28	Gerike & Gode (1990)[2]
APHA (1975)	67	% ThOD	20	Conway et al. (1983)[2]
Zahn Wellens (OECD 1992b)	>70	% DOC	7	Hoechst AG (1991a)
OECD (1992b) Method 302B	95	% DOC	20	Hoechst AG (1984a)
ISO (1994), Method 7827	94	% DOC	19	BASF AG (1996b)
OECD (2001), Method 303A	95	% DOC	28	Hoechst AG (1991b)

1 – Chemical concentration tested = 100 ppm

2 - Concentration of ethanedial tested is not provided, but is assumed to be 40% aqueous solution.

Although experimental degradation data are available for the 40% aqueous solution form of ethanedial, which primarily contains the dimer form, it is the hydrated monomer form which is expected to occur predominantly in the environment. Since no experimental data on the degradation of the hydrated monomer form of ethanedial are available, a QSAR-based weight-of-evidence approach (Environment Canada 2007) was also applied using the degradation models shown in Table 4b below. Given the ecological importance of the water compartment, the fact that most of the available models apply to water and the fact that ethanedial is expected to be released to this compartment, biodegradation in water was primarily examined.

Table 4b summarizes the results of available QSAR models for biodegradation in water. All of the modeled results show that the hydrated monomer of ethanedial will biodegrade quickly.

The biodegradation process of ethanedial (including identification of metabolites formed during a 28-day biochemical oxygen demand (BOD) study) has also been modeled using CPOPs (2008). Based on the model output, ethanedial will completely mineralize to CO_2

and water, without the formation of any stable metabolites. These modeled results agree well with the empirical results from the 28-day studies presented in Table 4a.

Using an extrapolation ratio of 1:1:4 for a water: soil: sediment biodegradation half-life (Boethling et al. 1995), the ultimate biodegradation half-life in soil is also <90 days and the half-life in sediments is <365 days. This indicates that ethanedial is not expected to be persistent in soil and sediment.

Table 4b. Modelled data for degradation of ethanedial (hydrated monomer form)

Fate Process	Model and model basis	Model Result and Prediction	Extrapolated Half-life (days OR hours)
WATER			
Primary biodegradation			
Biodegradation (aerobic)	BIOWIN 2008[1] Sub-model 4: Expert Survey (qualitative results)	4.23[2] "biodegrades fast"	≤ 182
Ultimate biodegradation			
Biodegradation (aerobic)	BIOWIN 2008[1] Sub-model 3: Expert Survey (qualitative results)	3.63[2] "biodegrades fast"	≤ 182
Biodegradation (aerobic)	BIOWIN 2008[1] Sub-model 5: MITI linear probability	0.98[3] "biodegrades fast"	≤ 182
Biodegradation (aerobic)	BIOWIN 2008[1] Sub-model 6: MITI non-linear probability	0.97[3] "biodegrades fast"	≤ 182
Biodegradation (aerobic)	CPOPs 2008 % BOD (biological oxygen demand)	1.0 "biodegrades very fast"	≤ 182

[1] EPIsuite (2008)
[2] Output is a numerical score from 0 to 5.
[3] Output is a probability score.

Based on the above empirical and modelled data (Tables 4a and 4b), ethanedial does not meet the persistence criteria in any medium (air, water, soil, sediment) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential for Bioaccumulation

A low modelled log K_{ow} value (<1) suggests that ethanedial has low potential to bioaccumulate in biota.

Since no experimental bioconcentration factor (BCF) or bioaccumulation factor (BAF) data for ethanedial were available, a predictive approach was applied using available BCF and BAF models as shown in Table 5 below.

BCF and BAF estimates, corrected for potential biotransformation, were generated using the BCFBAF model (EPI Suite 2008) and the Dimitrov sub-model (Dimitrov et al. 2005)

contained in the CPOPs model. Metabolic rate constants for the BCFBAF model were derived using structure activity relationships described further in Arnot et al. (2008a,b and 2009). Since metabolic potential can be related to body weight and temperature (Hu and Layton 2001; Nichols et al. 2007), the BCFBAFWIN model further normalizes the k_M for a 10g fish at 15°C to the body weight of the middle trophic level fish in the Arnot-Gobas model (184 g) (Arnot et al. 2008b). The middle trophic level fish was used to represent overall model output as suggested by the model developer and is most representative of fish weight likely to be consumed by an avian or terrestrial piscivore.

Kinetic mass-balance modelling is, in principle, considered to provide the most reliable prediction method for determining the bioaccumulation potential because it allows for metabolism correction (Arnot et al. 2008). According to the BCFBAF (2008) Helpfile, there is no universally accepted definition of model domain, however, biotransformation estimates may be less accurate for compounds outside the molecular weight and log K_{ow} ranges of the training set compounds. This is the case with ethanedial, since the estimated log K_{ow} value of ethanedial (-1.3) is outside of the log K_{ow} range (log Kow ~ -0.3 to 8.7) of the training set for submodels 2 and 3 of BCFBAF (2008), which include metabolism. Submodel 1 of BCFBAF (2008), which does not include metabolism, has a log K_{ow} training set range of -1.4 to 11.3. The results from the Dimitrov et al. (2005) model are considered to be acceptable, as the fragment coverage in the structural domain was equal to 67%.

Another model used (Table 5) is the one in the European Union Technical Guidance Document on Risk Assessment (European Commission 2003), which is based on the work of Veith et al. (1979). This model recommends a BCF value of 1.41 when the log K_{OW} is below 1.

Table 5: Modelled data for bioaccumulation in fish of ethanedial, hydrated monomer form

Model and model basis	Endpoint	Value wet weight (L/kg)	Reference
BCFBAF Sub-model 1: linear regression[1]	BCF	3.2	BCFBAF 2008
BCFBAF Sub-model 2: mass balance[2]	BCF	0.93	BCFBAF 2008
BCFBAF Sub-model 3: Gobas-mass balance[2]	BAF	0.93	BCFBAF 2008
Dimitrov, metabolism-corrected	BCF	2.3	Dimitrov et al. 2005
EU Technical	BCF	1.41	European Commission

Guidance Document			2003

[1] – no metabolism correction
[2] – includes metabolism correction

The available evidence indicates that ethanedial is expected to have low bioaccumulation potential. This is due to its physical and chemical properties, including its very low K_{OW} and very high water solubility as well as tissue biotransformation. Even without considering metabolism, this substance has a very low predicted BCF and BAF. Based on the available modelled values, ethanedial does not meet the bioaccumulation criterion (BCF or BAF ≥ 5000) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential to Cause Ecological Harm

Ecological Effects Assessment

There is experimental evidence that ethanedial has low toxicity to aquatic organisms following short-term (acute) exposures, which is consistent with findings of low bioaccumulation potential. Data also indicate that ethanedial has low toxicity to soil bacteria and at least one terrestrial plant species. Experimental ecological effects data are summarized in Table 6.

Table 6. Empirical data for aquatic and terrestrial toxicity.

Test organism	Test substance	Test duration	Endpoint	Value (mg/L)	Reference
Fish					
Zebrafish *(Danio rerio)*	no data	48 h	LC_{50}[1]	760-1100	Hoechst, Société Française (1983a)
Fathead minnow *(Pimephales promelas)*	no data	96 h	LC_{50}	215	Conway et al. (1983)
		48 h		230	
Orfe *(Leuciscus idus)*	40% aq.[2]	96 h	LC_{50} NOEC	460-680 316	Hoechst AG (1989a)
Turbot *(Rhombus maximus)*	40% aq.	96 h	LC_{50}	>500	Hoechst AG (1990b)
Invertebrates					
Daphnia magna	40% aq.	48 h	EC_{50}[3] EC_0	404 250	BASF AG (1988b)
Daphnia magna	no data	24 h	EC_{50}[3]	290-430	Hoechst, Société Française (1983b)
Algae					
Scenedesmus	40%. aq.	96 h	EC_{50}	>500	BASF AG (1988c)

subspicatus		96 h	EC_{20}	490	
Scenedesmus subspicatus	40%. aq.	72 h	EC_{50}	>250	Hoechst, Société Française (1993)
Pseudokirchneriella subcapitata[4]	no data	96 h	EC_{50}	149 (0-348)[5]	Bollman et al. (1989)
Bacteria					
Pseudomonas putida	40%. aq.	16 h	EC_{10}	46	Hoechst AG (1989b)
		16 h	EC_{50}	134	
Pseudomonas putida	no data	NR	EC_0	500	Gerike & Gode (1990)
Anaerobes (not characterized)	no data	24 h	EC_0 EC_{50}	200 625	Hoechst AG (1984b)
Terrestrial Plants					
Helian tuberosus (Jerusalem artichoke)	no data	NR	EC_{30}[6] NOEC	136 68	BUA (1997)

NR = Not reported

1 LC_{50} is the concentration of a substance that is estimated to be lethal to 50% of the test organisms

2 40% aqueous solution

3 EC_{50} is the concentration of a substance that is estimated to cause some toxic sublethal effect on 50% of the test organisms.

4 Formerly called *Selanastrum capricornutum*

5 This range represents the 95% confidence interval for the EC_{50} value.

6 Effect: inhibition of rhizome fragment proliferation

A robust study summary for the algae study by Bollman et al. (1989) (Table 6), which was used for derivation of the Predicted No Effect Concentration (PNEC) (see Characterization of Ecological Risk section), is included in Appendix I.

The ecotoxicity of ethanedial (hydrated monomer) was also modelled using ECOSAR (2008) and CPOPs (2008), and found to be very low. Ethanedial was modelled as a neutral organic by ECOSAR, and as a "base surface narcotic" by the OASIS sub-model in CPOPs. ECOSAR acute and chronic effect values ranged from 358 mg/L (for 14-day earthworm LC_{50}) to greater than 100 000 mg/L. The highest values were predicted for salt-water organisms. OASIS-CPOPs acute predictions for daphnid and fathead minnow were LC_{50}s of 77 000 and 63 000 mg/L, respectively. The modelled toxicity predictions show that ethandial (hydrated monomer) has low toxicity to aquatic organisms, which is in agreement with the empirical data.

Ecological Exposure Assessment

No data concerning concentrations of this substance in water in Canada have been identified; therefore, environmental concentrations are estimated from available information, including substance import, usage and release quantities submitted under S.71 of CEPA (Environment Canada 2010a), and size of receiving water bodies.

Some international data on concentrations of ethanedial in the environment are available. Ethanedial has been detected in surface and groundwater samples in the U.S. and Europe

at low (≤ 12 µg/L) levels (CICAD 2004). It has also been found in ozone-treated drinking water samples at a median concentration of 9 µg/L (IPCS 2000). More information on levels of ethanedial in the environment is available in the CICAD (2004) report.

A – Industrial Release

Aquatic Environment

Since a significant proportion of ethanedial is released to water (see previous section on Releases to the Environment), realistic but conservative (i.e. protective of the environment) scenarios were used to estimate concentrations of ethanedial resulting from industrial discharge. In these scenarios, ethanedial is released from industrial use to a wastewater treatment plant (WWTP) which discharges its effluent to a receiving water body. The concentration of the substance in the receiving water near the discharge point of the wastewater treatment plant is used as the predicted environmental concentration (PEC) in evaluating the aquatic risk of the substance. The PEC was calculated using the equations:

$$C_{water-ind} = \frac{1000 \times Q \times L \times (1-R)}{N \times F \times D} \quad [1]$$

$$PEC = C_{water\text{-}ind} + B \quad [2]$$

where

$C_{water\text{-}ind}$:	aquatic concentration resulting from industrial releases, mg/L
Q:	total substance quantity used annually at an industrial site, kg/yr
L:	loss to wastewater, fraction
R:	wastewater treatment plant removal rate, fraction
N:	number of annual release days, d/yr
F:	wastewater treatment plant effluent flow, m^3/d
D:	receiving water dilution factor, dimensionless
PEC:	predicted environmental concentration (from both industrial releases and natural sources), mg/L
B:	natural background concentration, mg/L

Exposure analyses were conducted for the aquatic compartment for the top six industrial users and/or importers of ethanedial in 2006, identified from respondents to the CEPA Section 71 Survey (Environment Canada 2010a). A combination of site-specific and generic exposure assumptions were used in the exposure analyses, to yield reasonable worst-case scenarios, as described below. Each user/importer reported an annual total use and/or import quantity of ethanedial in the range of 10 000 to 100 000 kg. These exposure analyses are therefore expected to represent realistic worst case release scenarios across Canada based on a general assumption that the quantity released is proportional to the quantity used, as per equation 1 above.

Dilution factors of 10 were applied for the concentrations in the receiving water, as all the sites discharged to large water bodies. A conservative WWTP effluent flow at the 10th percentile (3 456 m^3/d) of the WWTP discharge rates across Canada was used in the calculations. The scenarios assumed that the releases occur 250 days per year, typical for small and medium-sized facilities (a conservative assumption). The fraction lost to wastewater was based on either loss estimates reported by the company involved (Environment Canada 2010a) or a default loss estimate of 5% of the total quantity used by the company, whichever was greater. The generic loss estimate of 5% is based on conservative estimates for losses from container handling and cleaning (3%) (PEI 1988), transfer pipelines (1%) and process vessels (1%) (US EPA 1992). To take into account background concentrations of ethanedial naturally present in the water, a concentration of 0.01 mg/L was included in each PEC. This background concentration is very conservative, considering that ethanedial has been found in natural waters at concentrations of ≤ 12 μg/L (CICAD 2004).

WWTP removal rates after primary and secondary treatment of 90.2 %, 99.7 % and 96.0 % were predicted using the models SimpleTreat (1997), STP (2001) and ASTreat (2006), respectively (Environment Canada 2010c). These high predicted WWTP removal rates are due to the high rate of biodegradation that is predicted to occur during the secondary treatment phase. The predicted environmental concentration (PEC) calculation used the most conservative WWTP removal rate of 90.2 %, as modelled by SimpleTreat (1997), for those facilities with secondary treatment, assuming no removal (e.g., by degradation) in the receiving surface water. For facilities with only primary treatment, 0% removal by the WWTP was assumed.

Based on the above assumptions, the PECs were estimated to be in the range of 0.036 mg/L to 0.89 mg/L for the top 6 industrial users of ethanedial in Canada. The PEC values obtained are considered to represent the level of exposure under realistic worst case release scenarios in the receiving water near the point of the discharge from wastewater treatment plants.

Terrestrial Environment

Several companies reported releases of ethanedial to soil, including one paper products manufacturer, and two companies using ethanedial as part of a water-based drilling muds or fluids used for oil and gas exploration, at concentrations of 0.4 – 0.9% (Environment Canada 2010a). The releases to soil reported by the paper products manufacturers are relatively small (approximately 1% of their total usage of ethanedial) (Environment Canada 2010a).

One of the companies involved in oil and gas exploration reported that all waste from water-based drilling muds is disposed of using an Energy Resources Conservation Board approved borrow pit, “which is monitored and remediated to ensure sound environmental performance (Environment Canada 2010a)”. The other company involved in oil and gas exploration reported that their discharges of ethanedial in the drilling fluids to land (which totalled approximately 2000 kg in 2006) follow the regulations for discharge of

drilling fluids: "For land discharges, the drilling fluid system is tested with [a toxicity testing system using the bioluminescent marine bacterium (*Vibrio fischeri*) as the test organism]. If the drilling fluid passes [this bioassay], then it is land farmed. If it fails then it is sent to an offsite waste management facility. The products that have [ethanedial] would pass [the bioassay] themselves but other products in the drilling fluid system cause the drilling fluid to fail (Environment Canada 2010a)."

B – Consumer Release

Even though ethanedial is found in personal care products and in paints and coatings, it is expected to be found in only very small concentrations in these products, as a residual from polymerising reactions in the finished products (CNS 2010, Environment Canada 2010a). Ethanedial is also used in small concentrations (0.04-4%) in liquid disinfectants used for cleaning medical instruments such as in hospital facilities (DPD 2010). A total quantity of less than 1000 kg ethanedial was reported to be imported or used in disinfectants and other consumer products in Canada in 2006 (Environment Canada 2010a).

Quantities of ethanedial used in consumer applications are several orders of magnitude smaller than the amount used in industrial applications (Environment Canada 2010a). Therefore, since more dispersive releases from consumer use are expected to result in environmental exposure concentrations much smaller than those resulting from industrial applications, a PEC was not estimated for consumer releases.

Characterization of Ecological Risk

The approach taken in this assessment was to examine the available scientific information and develop conclusions based on a weight-of-evidence approach and using precaution as required under CEPA 1999. Lines of evidence considered include results from conservative risk quotient calculations, as well as information on the persistence, bioaccumulation, toxicity, sources and fate of the substance.

Ethanedial is expected to degrade relatively quickly in all environmental compartments and have low bioaccumulation potential. It also has relatively low ecotoxicity.

A conservative predicted no-effect concentration (PNEC) for the aquatic compartment was derived from the lowest aquatic toxicity value identified – the EC_{50} for algae (*Pseudokirchneriella subcapitata*) of 149 mg/L (Table 6). This value was selected as the critical toxicity value, and divided by an assessment factor of 10 to account for uncertainties related to interspecies and intraspecies variability in sensitivity, and extrapolation from a laboratory EC_{50} to a no-effect value in the field. This calculation resulted in a PNEC of 14.9 mg/L.

Risk quotients (PEC/PNEC) were calculated, based on the above-described PEC values for the top 6 industrial users/importers of ethanedial in Canada, and the PNEC of 14.9 mg/L. The risk quotients for the 6 sites ranged from 0.002 to 0.06. These conservative risk quotients for the aquatic compartment from the industrial scenarios described above indicate that exposure values are unlikely to be high enough to cause harm to aquatic organisms.

Since the majority of releases of this substance are likely to water at industrial manufacturing sites, and results of fugacity modeling indicate that virtually all of the substance discharged to water will remain in water, significant exposure of organisms at other types of locations or in media other than water are unlikely. In soil, ethanedial is not expected to persist (see Persistence Section), and given its low toxicity to soil bacteria and the one species of terrestrial plant tested, the above-described industrial releases of ethanedial to soil are not considered to be of concern.

Based on the above, ethanedial is thus unlikely to be causing ecological harm in Canada.

Uncertainties in Evaluation of Ecological Risk

It should be noted that this conclusion was reached despite the conservative assumptions that were made in response to uncertainties encountered in the assessment. A key uncertainty relates to the lack of empirical data on environmental concentrations in Canada, which was addressed by using conservative assumptions in the industrial exposure scenarios. There is also some uncertainty associated with the PNEC used in the risk quotient calculation, due to the paucity of chronic ecotoxicity data for this substance.

Given the use of this substance in other countries, it is possible that ethanedial is entering the Canadian market as a component of consumer products. Available information is currently not sufficient to derive a quantitative estimate that would help determine the importance of this source. Releases to sewer could be larger than the amount estimated here, due to widely dispersed releases from the imported consumer products.

Potential to Cause Harm to Human Health

Exposure Assessment

Environmental Media and Food

Only limited empirical data were identified for levels of ethanedial in environmental media.

Ambient air monitoring data for ethanedial is available, particularly for areas around large urban centers in North America and for the arctic (Liu 2006, Kawamura 1996, 2001, Weisel 2005). Studies from both Canada and the United States show detectable

levels of ethanedial in ambient air ranging between 0.00054 – 2.29 μg/m^3 (Liu 2006, Kawamura 1996, 2001). Canadian data from a monitoring site in Simcoe, Ontario, gathered in 1999-2000, were used to estimate the intake from ambient air. The mean concentration for ethanedial was 1.06 μg/m^3 (Aiello 2009). Other studies from the United States show similar concentrations with mean concentrations ranging from 0.49 – 1.81 μg/m^3.

Fewer studies were identified for indoor air monitoring; with no data available from Canada. Indoor air monitoring data was collected in two studies from the United States, comparing over 500 samples from three cities: Los Angeles, Houston and Elizabeth, NJ (Liu 2006, Weisel 2005). The mean concentrations during the sampling years 1999 – 2003 for ethanedial in each study were 0.87 μg/m^3 (Weisel 2005) and 2.53 μg/m^3 (Liu 2006). In the absence of Canadian indoor air data and given that there is a higher concentration of ethanedial in ambient air in the Simcoe Ontario study, 1.06 μg/m^3 was used as a surrogate for indoor air in estimating the environmental exposure.

For both soil and drinking water, no monitoring data was available in Canada or elsewhere. As a conservative approach, environmental concentrations for these media were estimated using ChemCAN, a Canada-specific environmental exposure model. Using submissions from section 71 reporting release amounts of ethanedial to the environment, predicted concentrations for water and soil were low to negligible – 8.17×10^{-3} μg/L and 1.19×10^{-3} μg/kg (Canada 2009b, ChemCAN 2003).

There is limited information on the concentration of ethanedial in the smoke of tobacco products available in Canada. However, Fujioka and Shibamoto (2005) detected ethanedial in tobacco smoke at concentrations of 1.93 to 6.98 μg per cigarette.
In a similar US study, the level of ethanedial in mainstream cigarette smoke from regular size cigarettes ranged from 1.93 to 4.78 μg per cigarette (Kazutoshi and Takayuki 2005).

There is limited information on the conetration of ethanedial in food. Heat processing reactions such as caramelization, roasting, frying or baking and the Maillard reaction of saccharides with proteins involving glucose contribute to the formation of ethanedial in various foods (Arribas-Lorenzo 2010, CICAD 2004). Studies have found low concentrations of the substance in food and beverages that have been fermented, baked or browned (SCCP 2005). Recent studies from Europe and Japan show the presence of ethanedial in various food products (Arribas-Lorenzo 2010, Weigel 2004, Barros 1999, Yamaguchi 1994). Naturally produced honey was found to contain 0.2–2.7 mg of ethanedial/kg of product (median: 1.7 mg/kg) (Weigel 2004). In a study of yogurt from Japan, ethanedial was detected at a concentration of 0.92 μg/kg (Yamaguchi 1994). In addition, fermented beverages, such as wine and beer, have been shown to contain concentrations of ethanedial ranging from 0.025 to 0.041 mg/L in beer and 0.36 to 1.5 mg/L in wine (Barros 1999, Yamaguchi 1994). The limited available data was used to estimate potential intake from food and beverages. While ethanedial is present in some fermented food products, fermented beverages are not commonly consumed by children under the age of 11. Therefore, beer and wine were removed from the intake estimate for this subpopulation.

The upper bounding estimate of daily intake for ethanedial from environmental media and food ranged from 0.3 µg/kg-bw per day (0–0.5 years old; breast-fed and formula fed) to 84 µg/kg-bw per day (0–0.5 years old; not formula fed).

Consumer Products

Ethanedial is used as an intermediate in the production of a number of compounds including resins and polymers, which have broad applications including paint coatings and primers, adhesives, paper resins and paper coatings.These uses may result in limited general population exposure to residual levels of ethanedial in certain consumer products (e.g., paint coatings and primers, paper). Upper-bounding estimates of exposure were derived for the following consumer product scenarios: inhalation and dermal exposure for individuals using water-based latex architectural paint and incidental mouthing of paper by young children.

Ethanedial is present as a production residual in cellulosic thickeners used for paint coatings and primers at concentration levels up to 0.5%. (Environment Canada 2010a, April 2010 email from Canadian Paint and Coating Association, to Risk Management Bureau, Health Canada; unreferenced). However, the concentration for ethanedial is lower in waterborne paint products due to dilution. Based on literature regarding the use of thickeners in paints, the total amount of thickener used would be between 0.4 and 1.8% by weight (June 2010 email from Canadian Paint and Coating Association, to Risk Management Bureau, Health Canada; unreferenced). This gives a conservative estimate of ethanedial upper-bounding concentration at 0.009% by weight in paint products. Ethanedial was not reported to be present in either industrial or automotive paint products. A conservative approach assumes that an individual's use of water-based architectural paint and primer containing 0.009% ethanedial is used once a year. The following scenario using the US EPA's Wall Paint Exposure Model was used to estimate the inhalation exposure. By priming and painting the walls of an enclosed room with a volume of 22 m^3 and an air flow rate of 0.45 air changes per hour, an acute inhalation exposure of 1.1×10^{-4} mg/kg-bw per event was determined assuming 20 hours of exposure to the air in the room (see Appendix III). This value is considered conservative based on the upper-bounding concentration of ethanedial used. As the function of ethanedial is to cross-link starches used in the thickeners, the compound is unlikely to be airborne. Furthermore, its low vapour pressure of 2.6 Pa (2.52×10^{-5} atm) indicates limited volatility.

A dermal exposure estimate to ethanedial for painters was derived using ConsExpo. The scenario was based on exposure of arms and hands to paint; painting of a surface area of 0.33 m^2, at a rate of 30 mg/min over 120 min, results in an estimate of acute exposure of 4.6×10^{-3} mg/kg-bw per event.

Submissions pertaining to the presence of ethanedial in paper products or paper coatings from several companies identified minimal levels (Environment Canada 2010a). Similarly, companies in Australia report low concentrations of ethanedial in paper

products (NICNAS 2004). The solvent form of ethanedial is used as an intermediate for paper and textile auxiliaries. In paper strength resins, ethanedial is polymerized with polyacrylamides allowing for an effective bonding with cellulose fibres (2010 personal communication from Forestry, Agriculture, Aquaculture Division, Environment Canada; unreferenced). As this is a wet treatment, it would be expected that a significant portion of free ethanedial would migrate to the wastewater stream. In the process of paper coatings, ethanedial is one of many paper coating additives, generally accounting for no more than 1% of the coating formulation. There may be little migration of ethanedial from the finished paper product due to the chemical binding of the additives and due to the desirable properties of the product containing ethanedial.

Incidental oral exposure to ethanedial could occur via mouthing of paper by young children. Such incidental exposure is more likely for children 0.5 – 4 years of age than for infants (0 – 6 months old). In a highly conservative approach for children aged 6 months to 4 years, oral exposure to ethanedial is estimated to be 0.24 mg/kg-bw per event assuming that ¼ of an 8.5 × 11" sheet of paper containing 0.37% w/w ethanedial is fully consumed, and all of the cross-linked ethanedial unlinks, solubilises and becomes bioavailable. This exposure estimate is considered very conservative as it is unlikely that all steps mentioned above will take place in reality for ethanedial to become bioavailable. Dermal exposure to ethanedial in paper is expected to be negligible for the general population.

Ethanedial may be detected as a residual from polymerization reactions in some cosmetic and personal care products; and is an intentional ingredient in a small number of cosmetic and personal care products used by the general population. According to Health Canada's CNS database, ethanedial is present at concentrations up to 0.1% in products such as skin cleanser, body and face moisturizer, hair dye, and shaving preparation. It is also present in hair conditioner up to 3% w/w and manicure preparation up to 1% w/w (CNS 2010). These may result in limited exposure to the general population. According to the European Union Health and Consumer Protection Directorate, maximum residuals levels of ethanedial in cosmetics may reach 100 ppm (SCCP 2005).

Ethanedial is also a nonmedicinal ingredient in one currently licensed natural health product, an acne facial cleanser at concentration of 0.001% w/w (LNHPD 2010). An acne face cleansing product sold in Canada with residual concentrations of 0.001% by weight (10 ppm) is used to estimate dermal exposure (April 2010 email from Natural Health Products Directorate, Health Canada, to Risk Management Bureau, Health Canada). Potential exposure to the facial area of an adult from daily use of acne face wash was estimated using ConsExpo, resulting in a chronic exposure of 7.05×10^{-5} mg/kg-bw per day (ConsExpo 2006) (see Appendix III).

The exposure estimates from use of cosmetics and personal care products were modelled based on the upper-bounding concentration reported by the CNS database (see Appendix III). The resulting daily dermal exposure estimates ranged from 3.6 μg/kg-bw per day (shaving preparation) to 0.23 mg/kg-bw per day (body lotion) for frequently used products, while the acute dermal exposure estimates for two products which are less

frequently used were 7.1 µg/kg-bw per event for manicure preparation product and 0.14 mg/kg-bw per event for hair dyes. The derived dermal exposure estimates are considered overestimates as they were based on the reported maximum concentration from CNS (CNS, 2010) Given that ethanedial is a residual in the ingredients used in cosmetic and personal care products, and that 0.1% is the maximum concentration in the lowest reportable range in the CNS where most of the products were notified in, the actual concentration level is expected to be much lower than 0.1%. This is further supported by the orders of magnitude difference in skin cleanser reported in both CNS (<0.1% w/w) and LNHPD (0.001% w/w). The concentration of ethanedial in manicure preparation would also be considered lower than what was reported by the CNS. Given that it is intended to cross-link nail protein, ethanedial is unlikely to be airborne or absorbed.

Inhalation exposure may also occur during use of manicure preparation products and Spray-on leave-in hair conditioner; however, the exposure to ethanedial during use of these products was estimated to be low (refer to Appendix III).

Ethanedial is present as an ingredient in pesticide and disinfectant products. Ethanedial is a formulant in pesticides. Pesticidal and disinfectant uses are limited to occupational settings. Therefore, exposure estimates were not derived for these products.

Uncertainty in Exposure Assessment

Confidence in the environmental media database is considered low due to lack of information. The Canadian ambient air data is considered acceptable for estimating air exposure to ethanedial. Despite the availability of indoor data from the United States, the Canadian outdoor air data was used, resulting in a more conservative estimate of exposure. In the absence of soil and water data, ChemCAN modelling was used to estimate Exposure, thereby increasing conservatism and uncertainty in the upper-bounding intake estimate.

Despite the lack of data on levels of ethanedial in food in Canada and the limited data available for food products containing ethanedial elsewhere, it was considered appropriate to assess the potential for Canadians to be exposed to ethanedial in foods using the available data. The resulting dietary intake estimate may overestimate exposure and is not considered representative of the Canadian situation.

Confidence in the estimates of exposure to products available for consumer use is considered moderate. There is little uncertainty in the concentrations of ethanedial found in consumer products; however, uncertainty exists due to conservative inputs in the exposure models, particularly those consumer products reporting a range <0.1% from CNS and in the paper mouthing scenario where it is assumed that all of the crosslinked ethanedial is bioavailable and absorbed.

Health Effects Assessment

A summary of the available toxicological information for ethanedial (glyoxal) is presented in Appendix IV.

The European Commission (EU) has classified ethanedial as a category 3 mutagen (*causes concern for humans owing to possible mutagenic effects*) based on predominantly positive results for mutagenicity in *in vitro* and equivocal results in *in vivo* assays (EU 1996). The results of *in vitro* assays of genotoxicity are relatively consistent. In bacteria, ethanedial induced reverse mutations in multiple *Salmonella* strains in the Ames' assay, and in various strains of *E. coli* (WP2uvrA, pKM101) with and without metabolic activation depending on strain (summarised in OECD 2003). Ethanedial was also positive in multiple bacterial assays for DNA damage including the SOS *umu*, SOS chromo, and *rec* assays (Von der Hude et al. 1988; Matsui et al., 1989; Ono et al. 1991a,b). However, ethanedial was negative in host-mediated assays (mouse, intravenous exposure) for bacterial DNA damage (Hellmer & Bolcsfoldi 1992b).

Ethanedial exposure produced predominantly positive results in *in vitro* mutagenicity assays in mammalian cell lines. Positive results were obtained in the mouse lymphoma L5178TK$^{+/-}$ forward mutation assay without activation, and in COS-7 cells carrying the pMY189 plasmid, which was treated with glyoxal prior to its introduction into the cells (Wangenheim & Bolcsfoldi 1988; Murata-Kamiya 1997b). Reversion of CHO AUXB1/GAT cells to adenosine-thymidine-glycine prototrophy was also observed *in vitro* (Taylor & Wu 1980; Taylor et al. 1984). However, ethanedial did not induce forward mutations at the HPRT locus in CHO-S/HPRT or V79/HGPRT cells (Taylor et al. 1983; Societe Francaise Hoechst 1986a). Chromosomal aberrations were observed in Chinese hamster ovary (CHO) cells and in V79 cells with and without metabolic activation (Henkel 1986; Nishi et al. 1989). CHO AUXB1, CHO (normal), and human lymphocytes were all positive for sister chromatid exchange after exposure to ethanedial (American Cyanamid Co. 1982; Tucker et al. 1989). Furthermore, *in vitro* investigations of DNA damage indicated that ethanedial exposure increased instances of unscheduled DNA synthesis and single-strand breaks in Syrian hamster TC-SV40 cells and mouse lymphoma L5178TK$^{+/-}$ cells, respectively (Cornago et al. 1989; Garberg et al. 1988; Wangenheim & Bolcsfoldi 1988). Ethanedial did not induce DNA cross-links in rat hepatocytes in the presence of a metabolic activation system (Ueno et al. 1991b), but did cause increased DNA damage in TK6 human lymphoblastoid cells exposed to ethanedial (Henderson et al. 1998). At higher doses, DNA damage was also detected in rat hepatocytes by comet assay (Kuchenmeister et al. 1998). Interestingly, a dose-dependent decrease in the tail moment, and an increase in highly condensed, circular DNA spots were observed as dose increased. Of 100 compounds tested by this method, this type of damage appeared to be specific for certain aldehydes (WHO 2004). The authors suggested that the cross-linking ability of glyoxal may have prevented DNA from migrating through the gel at higher doses (Kuchenmeister et al. 1998); however, no data was provided to support this conjecture. Furthermore, human umbilical vein endothelial cells incubated in the presence of 100 µg ethanedial /ml had significantly increased numbers of formamidopyrimidine *N*-glycosylase (FPG)-sensitive sites without a concomitant increase in intracellular hydroperoxides (Shimoi et al. 2001). FPG is known to repair oxidative DNA damage and abasic sites as well as repair guanine-glyoxal

adducts (WHO 2001), thus suggesting that ethanedial reacts directly with DNA intracellularly. In an earlier report, cell transformation was not observed when mouse embryonic cells were exposed to ethanedial (Mason 1980a, b, c) (see Appendix IV for details).

The genotoxicity of ethanedial has also been investigated *in vivo*. Swiss mice exposed orally to ethanedial did not have an increased incidence of micronuclei in the bone marrow at time-points up to 72 h (Societe Francaise 1986b). In an older, insufficiently documented study, subcutaneous injections of ethanedial caused chromosome aberrations in the duodenum, testes, and spleen in rats. No increase in the incidence of chromosomal aberrations was observed in the liver or pancreas (Thomas 1958). Unscheduled DNA synthesis was observed in the pyloric mucosa, but not in primary rat hepatocytes of rats exposed by gavage to ethanedial (Furihata et al. 1985; CCR 1992). Additionally, exposure by gavage, resulting in high localized concentrations, caused dose dependent increases in the number of DNA strand breaks observed in the pyloric mucosa and livers of exposed rats (Furihata & Matsushima 1989; Ueno et al. 1991b). Ethanedial was negative in the sex-linked recessive lethal mutation test, the dominant lethal test, and for reciprocal translocations in *Drosophila melanogaster* (Barnett & Munoz 1989).

The mechanism of ethanedial induced mutagenicity *in vitro* is not completely understood. Ethanedial has been shown to form a cyclic ethanedial dG-adduct when incubated with purified DNA under physiological conditions, *in vitro*. The investigators also detected GC and GA cross-links in glyoxal treated DNA (Kasai et al. 1998). Other studies have confirmed that ethanedial can form stable adducts with DNA *in vitro* (summarised in OECD 2003). However, Ueno et al (1991) demonstrated, in *Salmonella typhimurium*, that the observed mutagenicity is not necessarily due to direct interaction of glyoxal with DNA. Rather, the generation of free radicals was determined to be the cause of the observed mutagenicity, with the singlet oxygen species being the most important. Treatment of the bacteria with chemicals that scavenge singlet oxygen almost completely suppressed mutagenesis induced by glyoxal treatment (Ueno et al. 1991c). Therefore, it is unclear whether the genotoxicity of glyoxal, observed in some *in vivo* studies, is due to penetration of ethanedial across the cell membrane and the nuclear envelope and direct interaction with the DNA or by triggering the formation of DNA-damaging free radicals such as singlet oxygen.

Ethanedial has not been classified by other international agencies or regulatory bodies as to its carcinogenicity. No chronic toxicity or carcinogenicity data is available with respect to the oral or inhalation exposure routes. The effects of chronic dermal exposure were investigated in a lifelong bioassay in mice. Groups of mice were exposed topically to one of two commercial preparations of ethanedial for a total exposure of 23 mg/kg-bw/day[c] for life. Control mice were treated with deionised water. Ethanedial did not induce tumours on the skin of any mice. One male receiving European ethanedial 40 developed a fibrosarcoma, which was not considered related to treatment as historical data indicated that these often occur spontaneously in males of this strain (Bushy Run 1982).

[c] Doses converted using Health Canada reference values (Health Canada 1994).

Ethanedial has been tested in both tumour-initiating and -promoting assays. CD-1 mice exposed topically to approximately 485 mg ethanedial/kg-bw/day (1698 mg/kg-bw administered twice weekly) for 5 weeks, followed by 47 weeks of dermal exposure to 12-O-tetradecanoylphorbol-13-acetate (promoter), did not have increased incidences of skin tumours when compared to controls (Miyakawa et al. 1991). Wistar rats exposed via drinking water to *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) for 8 weeks, followed by 32 weeks of exposure to approximately 907 mg ethanedial/kg-bw/day, had significantly ($p<0.05$) increased incidences of adenocarcinomas and hyperplasias of the stomach when compared to those that did not receive ethanedial (Takahashi et al. 1989) demonstrating a tumor-promoting potential *in vivo*.

No developmental studies via the dermal or inhalation route were identified. In contrast, oral dosing developmental studies conducted by the United States National Toxicology Program (NTP) were identified. In a range-finding study, SD rats were exposed orally, by gavage, from GD 6-15, to ethanedial trimeric dihydrate (the predominant form in solution), which converted to 166 to 1657 mg/kg-bw/day ethanedial. Maternal toxicity was evidenced by decreased weight gain at the 166 mg/kg-bw/day level and above. At exposures greater than 663 mg/kg-bw/day, clinical signs of toxicity and decreased gravid uterine weight were observed. Maternal deaths occurred at 994 mg/kg-bw/day and above (NTP 1991c). The follow up to the range-finding study exposed SD rats orally to 41, 124, 249 mg/kg-bw/day ethanedial (converted from ethanedial trimeric dihydrate) from GD 6-15. No maternal toxicity or embryotoxic effects were observed at any level in this study. A more recent investigation was conducted using 19-24 female Wistar rats per group. The rats were exposed by gavage to 0, 5, 25, or 125 mg/kg-bw/day ethanedial between GD 6 – 19. Maternal toxicity occurred only at the highest dose while no effects were observed on any developmental parameters (BASF & Clariant 2001). Based on these findings an oral LOAEL of 125 mg/kg-bw/day was derived for maternal toxicity, and an oral NOEL of >125 mg/kg-bw/day was derived for developmental toxicity, in rats.

Oral developmental studies conducted in rabbits indicated that maternal toxicity occurred only at concentrations that were considered corrosive and damaging to the gastric mucosa. New Zealand white rabbits receiving 0, 166, 331 mg/kg-bw/day ethanedial, by gavage, experienced systemic toxicity, decreased weight, and decreased foetal weight at the high dose only. No effects were observed at exposures below the high-dose (NTP 1991d, 1992). From this study oral LOAELs of 331 mg/kg-bw/day were derived for both maternal and developmental toxicity in rabbit.

No reproductive studies were identified. However, the results of several -dose studies, summarised below, indicate that ethanedial does not affect the male reproductive tract except at very high doses. In a 90-day oral exposure (drinking water, 107, 234, 315 mg/kg-bw/day) toxicity study with male rats a dose-dependent decrease in the absolute weight of all organs except for the testes and brain was observed. There was also no change in the weight of the testes when rats were exposed by the same route for 180 days to a dose of 298 mg/kg-bw/day (Ueno et al. 1990a). Another 90-day toxicity study exposed male and female rats to ethanedial in the feed at doses up to 250 mg/kg-bw/day. No macroscopic or histopathological changes were noted in any organs in any dose

groups (Mellon 1966). In a 90-day drinking water study, male rats (10/group) were exposed at levels up to approximately 2286 mg/kg-bw/day. At the highest-dose tested male rats experienced hypospermia in the epididymis with atypical cells and slight degenerative changes in the germ epithelium in the testes. It should be noted that at this dose all animals were removed from the study after 2-weeks, due to severe cachexia. The German Advisory Committee on Existing Chemicals suggested that the effects on the male reproduction system may have been due to cachexia of the animals due to decreased water intake (NTP 1991a, BG Chemie 1998).

Decreased body and organ weights and decreased food intake were the most consistently observed effects in rats, exposed by the oral route, in repeated-dose, short-term and sub-chronic bioassays. CrlCD(SD)Br rats (6/sex/group) were exposed to 40, 120, or 400 mg/kg-bw/day ethanedial via drinking water in a 28-day bioassay. Body weight gain was decreased by 8% and 2.5% in mid dose males and females, respectively, and by 32% and 15% in high-dose males and females, respectively. Food intake was decreased by 8% and 33% in males of the 120 and 400 mg/kg-bw/day groups, respectively. In females, the food intake was only decreased in the 400 mg/kg-bw/day group (18%). Water consumption also decreased in a dose-dependent manner. Authors noted that this may have been due to the palatability of the test substance. No effects on biochemical, haematological, or urinary parameters were identified in this study (Societe Francaise Hoechst 1987). An oral short-term LOAEL of 120 mg/kg-bw/day was identified in this study based on the decreases in body-weight gain and food intake.

Several sub-chronic investigations examined the effects of oral exposure to ethanedial on male SD rats. In one investigation male rats were exposed in the range of 107 – 451 mg/kg-bw/day (see Appendix IV), via the drinking water for 30, 60, 90, or 180 days in two phases (Ueno et al. 1991a). The first phase exposed rats to three doses for 30, 60, or 90 days, while the second phase exposed rats to the high dose only for 90 or 180 days. It should be noted that total exposure decreased in each exposure group as duration of exposure increased (i.e. – the low-dose for the 90 day group is lower than that for the 30 or 60 day groups etc.). The decrease in total exposures was probably due to decreased water consumption as dose and duration of exposure increased, which was likely related to the palatability of the test substance. Commonly observed effects were significant decreases in water consumption in the low dose groups and in body weight, and food and water consumption in the mid- and high-exposure groups. The absolute weights of livers, kidneys, hearts and spleens were decreased in a dose-dependent manner in all dose groups and exposure periods, and were significantly different than controls in the mid- and high-dose groups. Relative kidney weights were increased at the high-dose after 90 days compared to controls. After 180 days of exposure to the high-dose, relative liver, kidney, and heart weights were increased in comparison to controls. Biochemical analyses identified a significant decrease in lactate dehydrogenase (LDH) in the high-dose group, significant decreases in alanine and aspartate aminotransferase (ALT, AST), albumin, and total protein in the mid- and high-dose groups, and a significant reduction in total serum protein in the low-dose group of the rats exposed for 90-days. In the 30 and 60 day groups, ALT was significantly decreased in all exposure groups. After 180 days, AST was only slightly reduced while ALT and LDH levels were unaffected; however,

total serum protein content was decreased significantly in the 180 day exposed animals. Furthermore, declines in the incorporation of L-[^{3}H]leucine were observed in rats treated either intravenously (150 mg/kg- bw), or orally (1000 mg/kg-bw), with ethanedial as compared to those not receiving ethanedial, indicating that ethanedial does inhibit protein synthesis. In the 30-day bioassay, reductions in glyoxalase I and II activity were observed. However, levels of these enzymes were normal in the longer exposure groups. There were also no effects observed on levels of glutathione or 2-thiobarbituaric acid-active substances in the liver, kidney, or erythrocytes. After 180 days exposure to 298 mg/kg-bw/day, 2 animals experienced polyps and haemorrhage in the forestomach, which the authors did not consider to be related to test substance exposure. Additionally, 4 animals displayed slight swelling of the papillary epithelial cells in the kidneys, papillary edema, and congestion of the lymph nodes located near to the kidneys (Ueno 1991a).

A second investigation exposed Wistar rats to 40% ethanedial in the feed at concentrations of 32, 63, 125, or 250 mg/kg-bw/day for 90-days. Significant reductions in body weight were observed after 2 weeks, but were reversible. In contrast to the studies outlined above, absolute liver and kidney weights were increased in the high dose group. No examinations were carried out to assess macroscopic or micropathological effects on other organs. No biochemical or haematological parameters were investigated (Mellon Institute 1966). Similar effects were observed in other sub-chronic investigations of ethanedial toxicity (Summarised in Appendix IV).

From the aforementioned sub-chronic studies, a sub-chronic oral LOAEL of 107 mg/kg-bw/day is derived based on decreased total serum protein levels in the male SD rat. Decreased water consumption was also observed at this dosage, but, as mentioned above, was likely related to the palatability of the test substance. The authors of the study from which this oral LOAEL was derived concluded that sub-chronic exposures to glyoxal resulted in an overall low degree of systemic toxicity (Ueno et al. 1991a).

Exposure by the inhalation route was examined in one short-term bioassay. Wistar rats (5/sex/group) were exposed (nose-only) to nominal concentrations of 0, 0.4, 2, or 10 mg/m^3 ethanedial, with mean aerodynamic mass diameters of 0.8 – 1.2 µm, 6 hours per day, 6 days per week, for 29 days. At the mid- and high-exposure concentrations animals displayed minimal squamous metaplasia of the epiglottal epithelium in the larynx accompanied by sub-mucosal lymphoid cell infiltration. No other adverse events were noted. Based on these findings a short-term LOAEC of 2.3 mg/m^3 (Average measured concentration at the nominal 2 mg/m^3 dose) was derived based on local effects in the larynx (Hoechst 1995).

A ten-day range finding study was carried out to determine suitable dermal exposure levels for a chronic exposure bioassay in mice. Mice were exposed to 53 or 105 mg/kg-bw/day each day for ten days. At the highest dose, open sores formed on the skin. No other adverse effects were observed in this study (Bushy Run 1982). From this study a LOAEL of 105 mg/kg-bw/day was derived for site of contact effects following short-term dermal exposure in mice. No sub-chronic data was available to address dermal exposures.

Ethanedial is an irritant and a sensitizer. It is classified by the European Union as R36/38 (*irritating to eyes and skin*) and R45 (*May cause sensitization by skin contact*) (ESIS 2010). Positive results were reported for skin and mucous-membrane (eye) irritation in white rabbits in several assays (Smyth et al. 1962; BASF AG 1963a,b; Ito 1963; BASF AG 1985b,c; WHO 2004). Skin irritation appeared to be related to the concentration of the test substance and the length of exposure. Sensitization was investigated in both humans and guinea pigs. In humans, 24 volunteers were exposed to patches soaked with 10% ethanedial, 5 times for 48 hrs each time. Twenty-four hours after the final application of the induction phase, volunteers were exposed to 2% ethanedial, under occlusive conditions, for 48 hours. All volunteers experienced reactions (Kligman 1966). In a second human investigation, workers with occupational dermatitis, suspected to be due to contact with ethanedial 40, were patch tested with 20% ethanedial. Seven of nine workers tested had positive reactions to the patch testing (Ito, 1963). Exposure to "neat" ethanedial, in its powder form, caused no irritation nor did it elicit a sensitization reaction (Monsanto Co. 1969). Ethanedial was positive in both the maximization and Buehler tests in guinea pigs (BASF AG 1987; American Cyanamid Co. 1988).

Detoxification and Metabolism of Endogenous Ethanedial

Endogenous formation of ethanedial occurs within the cellular milieu through several non-enzymatic reactions such as the Maillard reaction (which occurs between sugars and amino acids), DNA oxidation, peroxidation of polyunsaturated fatty acids, and UV photo-damage (Fig. 1). Additionally, conditions of oxidative stress and GSH depletion as well as the metabolism of glycoaldehydes, ethylene oxides, and β-hydroxy-substituted *N*-nitrosoamines can contribute to the formation of ethanedial *in vivo* (WHO 2004).

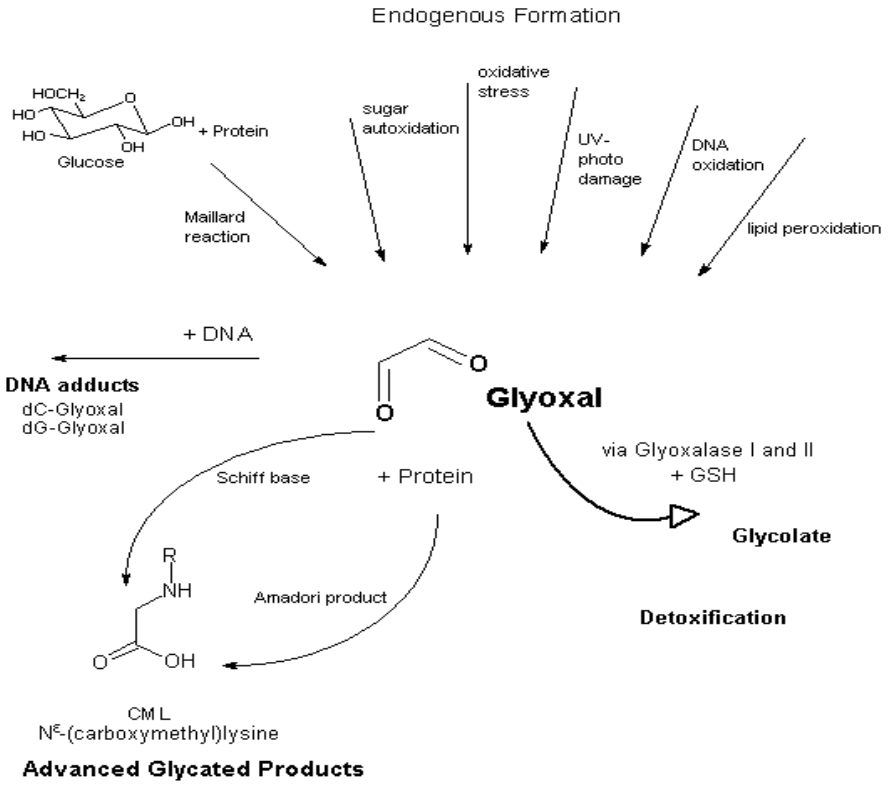


Fig. 1: Endogenous Formation and Detoxification of Ethanedial (Glyoxal) (Concise International Chemical Assessment Document 57, WHO 1994)

Ethanedial (Glyoxal) is efficiently detoxified within the body by rapid reaction with proteins, and by a specialized glyoxalase system. Glyoxal reacts non-enzymatically with GSH with formation of a hemithioacetal, which is subsequently converted to S-glycolylglutathione by glyoxalase I. Glyoxalase II catalyses the hydrolysis of S-glycolylglutathione to glycolate (a nongenotoxic substance), re-generating the GSH from the first reaction. Therefore, the intracellular concentration of GSH plays a key role in determining the activity of the glyoxalase system (Thornalley 1995; Sady et al. 2000. International Programme on Chemical Safety's Concise International Chemical Assessment Documents. Number 57: Glyoxal (2004)). Other enzymatic pathways can also contribute to ethanedial detoxification. These include the NADPH-dependent aldose reductases, aldehyde dehydrogenases (ALDH), and 2-oxoaldehyde dehydrogenase (Kuhla et al. 2005). Thus, there are a number of protective systems to ensure any potential ethanedial (Glyoxal) hazard is neutralized.

Under normal conditions, because of binding to macromolecules (ethanedial reacts with the arginine, cysteine, and lysine side-chains of proteins), very little free ethanedial is present in biological systems. This mechanism results in the reversible binding of greater than 90% of ethanedial in biological systems, leaving less than 10% of ethanedial free within the cell (Thornalley 1995).

No quantitative data is available for the absorption, distribution, metabolism, or excretion of exogenous ethanedial in either human or animal models. A concentration of 13.2 µM ethanedial was reported in normal human urine (number of subjects not reported) (Espinosa-Mansilla et al. 1998; WHO 2004). The blood plasma concentration of ethanedial has been measured in 'normal' patients and those with certain pathological conditions (diabetes mellitus, uraemia). Ethanedial concentrations were increased up to two fold in diabetics (Thornalley 1998, 2000). Agalou et al. measured concentrations of ethanedial in the blood plasma in healthy subjects, patients with mild to moderate uraemia, and patients with end-stage renal disease receiving haemodialysis and determined them to be 0.23±0.13 µmol/L (n=6), 0.4±0.16 µmol/L (n=10), and 0.76±0.21 µmol/L (n=5), respectively (Aagalou et al. 2002). A second study reported blood plasma concentrations of 0.3 µmol/L for healthy subjects (n=3), 0.45 µmol/L for patients with poorly controlled diabetes, and 0.47 µmol for patients with chronic renal failure (Lapolla et al. 2003). Thus, it is evident that diabetics and patients with renal failure can have increased levels of ethanedial present in the blood.

Characterization of Risk to Human Health

Based principally on its classification as a category 3 mutagen by the European Commission (EU 1996), genotoxicity is considered to be a critical effect for the characterization of risk to human health for ethanedial. Ethanedial was mutagenic in a range of *in vitro* assays and caused DNA strand breaks and increased unscheduled DNA synthesis in *in vivo* assays. However, these were confined mainly to the pyloric mucosa and liver, were statistically significant only at high doses delivered by gastric instillation, and did not occur in organs distant from the site of contact. Furthermore, rats exposed to drinking water containing ethanedial for up to 180 days did not display gastrointestinal effects, indicating that only with single concentrated doses do gastrointestinal effects become evident and that the method of animal dosing plays a role in the observed effects of ethanedial exposure. Rabbit studies performed by gavage confirm these observations, reporting corrosiveness in the GI tract in animals exposed to levels above 166 mg/kg-bw/day (mid-dose).

Protection from the potential effects of ethanedial exposure is provided by the glyoxalase system. The glyoxalase system is present within the cytosol of all cells and is known to be very efficient in reducing the amount of carbonyl stress caused by α-oxoaldehydes such as ethanedial. This efficiency is partly due to the system's narrow substrate specificity, which detoxifies ethanedial and methylglyoxal almost exclusively (Thornalley 1994, 2003). In order for ethanedial to exert genotoxic effects *in vivo*, it would be necessary for levels to reach an intracellular concentration that could overwhelm protein sequestration and detoxification by the glyoxalase system. Other enzymes and enzymatic systems are also known to detoxify glyoxal including the NADPH-dependent aldose reductases, aldehyde dehydrogenases (ALDH), and 2-oxoaldehyde dehydrogenase (Kuhla et al. 2005).

Additionally, ethanedial is a potent cross-linker, and it is therefore likely that exposure to ethanedial at levels the general population may be expected to be exposed to, would not be readily absorbed, but would become bound to macromolecules at points of entry thereby preventing ethanedial from coming into contact with the genetic material.This in itself is considered to act as a protective barrier, preventing the manifestation of genotoxic effects. In *in vitro* assays, in which ethanedial tests positive for genotoxicity, individual cells do not have this protective barrier and are subject to relatively large concentrations of test substance that can penetrate cell membranes and interact with DNA. Thus, genotoxic effects are observed.

The carcinogenicity of ethanedial has not been investigated in oral or inhalation bioassays, and no data was identified regarding ethanedial's carcinogenicity in humans. Mice exposed to ethanedial dermally for their entire lifespan did not have an increased incidence of neoplasias. Additionally, no pre-neoplastic changes were observed in oral studies of shorter lengths (6 months). An increase in the number of adenocarcinomas of the stomach was observed when rats were pre-treated with the known carcinogen *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG) before oral exposure (drinking water) to approximately 907 mg ethanedial/kg-bw/day for 32 weeks, indicating that ethanedial may act as a tumour promoter. However, no tumours were evident in the stomachs of rats receiving glyoxal only. Another straight chain unsaturated dialdehyde, pentanedialdehyde, has a similar mutagenic profile to ethanedial but was not considered carcinogenic in 2-year inhalation bioassays in F344 rats and B6C3F1 mice by the US National Toxicology Program (van Birgelen 2000). Ethylene glycol, a substance that is metabolized to glyoxal by oxidative dehydrogenation, (Kurina et al. 1998) was not carcinogenic in a 2-year oral feeding study in male and female mice (NTP 1993).

As discussed, efficient detoxification of ethanedial by the glyoxalase system, other enzymatic systems, and protein binding of free ethanedial are considered to prevent ethanedial from interacting with DNA within tissues distant from the points of contact. Although it is conceivable that tissues present at points of contact may have some susceptibility to the genotoxic effects of ethanedial, the absence of carcinogencity via the dermal route indicates this may not be relevant from a human health perspective. Very high exposures could potentially overwhelm systems and lead to the presentation of adverse effects. For example, the glyoxalase system is dependent on GSH as a catalyst for the detoxification of ethanedial. During very high exposures, GSH could be depleted leading to the accumulation of free ethanedial, which in turn could result in increased DNA damage, and formation of AGEs. However, considering the protective barriers in place, it is considered that ethanedial concentrations must overwhelm the glyoxalase system, protein binding and additional pathways of enzymatic detoxification in order to induce genotoxicity *in vivo*. Therefore, it is considered that ethanedial levels are required to surpass a threshold in order to induce genotoxic effects.

For all age groups, except infants fed breast milk or formula, the principle route of exposure to ethanedial is expected to be from the daily intake of food and beverages. With respect to non-genotoxic effects, comparisons of the upper-bounding estimates for total daily intake (0.3 to 84 µg/kg-bw per day) to the lowest non-cancer LOAEL for repeated exposure via the oral route of 107 mg/kg-bw per day (for reduced serum protein

levels) results in margins of exposure (MOEs) ranging from 1 300 to 360 000. As a worst case scenario, the European Union proposed an intake estimate from food of 10 µg/day, or 160 µg/kg-bw (64 kg) per day from all environmental media, which is less conservative than the approach in this assessment despite the large concentration found in food (SCCP 2005). However, considering that some of the food data is not reflective of dietary needs, but rather occasional food items (e.g. beer and wine), it is likely that the actual margin of exposure for the general Canadian population is greater than those derived in this assessment.

Estimates of oral exposure are considered to be worst-case scenarios that overestimate the actual exposure of the general population to ethanedial and resulting margins of exposure are considered to be protective of non-cancer effects. While incidental oral exposure could theoretically occur via paper mouthed by young children, it is unlikely that ethanedial becomes bioavailable during such events.

Genotoxicity was observed in rats exposed orally, by gastric instillation; however, in the 90-day study from which the oral LOAEL of 107 mg/kg-bw per day was identified, no GI tract effects were noted. Additionally, genotoxicity was only statistically significant at doses several fold higher than the oral LOAEL and was only observed when ethanedial was administered acutely as a concentrated bolus dose, which is not reflective of the nature of exposures that could occur in the general population. Based on these observations and information on the metabolic fate of ethanedial, it is considered that the range of margins of exposure based on the comparisons of the oral LOAEL of 107 mg/kg-bw/day to the estimates of oral exposure are also protective against genotoxic effects in the human population.

Exposure to ethanedial via the dermal and inhalation routes could also occur within the general population. Based on consumer product modelling, the air concentration of ethanedial resulting from painting a room area was 0.11 µg/m^3. Because painting is considered to be an acute exposure for the general population, the estimated exposure of 0.11 µg/m^3 was compared with the acute (7-hour) LOEC for inhalation of 4200 mg/m^3 (Appendix IV), which is based on an observed increase in breathing rate. This comparison results in a margin of exposure of 3.8×10^7. The comparison of the dermal LOAEL of 105 mg/kg-bw per day (for local skin necroses) with the dermal exposure from paint splatter, 4.6 µg/kg-bw per day, gives a margin of exposure of 23 000.

The chronic exposure estimates from use of cosmetics and personal care products, including acne face wash, ranged from 0.071 µg/kg-bw per day (face wash) to 0.27 mg/kg-bw per day (body lotion). Comparing the short-term dermal effect level of 105 mg/kg-bw per day with these exposure estimates yields margins of exposure ranging from 390 to 1 500 000. When these exposures are compared to the single exposure administered to mice for their entire life-span (23 mg/kg-bw/day), for which no statistically significant effects were observed, margins of exposure ranging from 85 – 324 000 are derived. It should be noted that the estimated exposure from acne face wash is based on actual product concentrations of ethanedial, while the estimate for body lotion, and other cosmetics, are based on the maximum value within the reporting range from

CNS, which is considered to be an overestimate. Therefore, the low end of the range of margins of exposure is considered to be much higher than reported here. The acute exposure estimates from use of hair dyes and manicure preparation products were 0.14 mg/kg-bw per event and 7.1 μg/kg-bw per event, respectively. Comparing the short-term dermal effect level of 105 mg/kg-bw with these exposure estimates yields margins of exposure of 750 and 15 000, respectively.

Comparing the acute (7-hour) LOEC for inhalation of 4200 mg/m^3 with the estimated mean event concentrations during use of of spray-on leave-in hair conditioner (0.04 mg/m^3) manicure preparation products (0.42 μg/m^3) yields margins of exposures of 105 000 and 1 x 10^7, respectively.

These margins of exposure are considered adequate to address uncertainties in the health effects and exposure databases.

These margins of exposure are considered adequate to address uncertainties in the health effects and exposure databases.The margins of exposure derived above are all based on comparisons of conservative, upper-bounding estimates exposure of the general population to the critical effect levels identified for oral, inhalation, and dermal exposures of the appropriate durations. The derived margins of exposure are considered to be adequate to address uncertainties in the health effects and exposure databases.

Uncertainties in Evaluation of Risk to Human Health

The scope of this screening assessment does not include a full analysis of the mode of action of ethanedial, nor does it take into account possible differences between humans and experimental species in sensitivity or potential differences in toxicity due to route of exposure. Furthermore, there is uncertainty in the assessment due to the lack of data available to determine the carcinogenicity of this substance following chronic oral and inhalation dosing. There is also uncertainty concerning the effects of ethanedial exposure on reproductive toxicity, as the available information was limited to examinations of reproductive organs after shorter-term repeated-dose studies, although no ill-effects were observed on these organs except at exceedingly high exposure levels. There is moderate confidence in the database concerning non-cancer effects as there is data available that addresses, short-term, and sub-chronic effects induced by exposure through the oral route, and short-term effects induced by the inhalation route. Longer-term studies were not available for the inhalation route. There are also adequate studies that address developmental toxicity induced by the oral exposure route.

There is low confidence in the environmental media data concerning water, air, soil and food intakes due to the lack of available Canadian data and the requirement for environmental modelling via ChemCAN. Confidence in consumer products, however, is moderate due to the availability of data for ethanedial concentrations found in products. As modelled, the exposures from products are considered to be overestimates.

Conclusion

On the basis of the adequacy of the margins between upper-bounding estimates of exposure to ethanedial and critical effect levels, it is concluded that ethanedial is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

Based on the information presented in this final screening assessment, it is concluded that ethanedial is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends. Additionally, ethanedial does not meet the criteria for persistence and bioaccumulation potential as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

It is therefore concluded that ethanedial does not meet any of the criteria under section 64 of CEPA 1999.

This substance will be considered for inclusion in the Domestic Substances List inventory update initiative. In addition and where relevant, research and monitoring will support verification of assumptions used during the screening assessment.

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Appendix I: Toxicity to Algae

Reference: Bollman MA, Baune WK, Smith S DeWhitt K, Kapustka L. 1989. Report on algal toxicity tests on selected Office of Toxic Substances (OTS) chemicals. Corvallis, OR, US Environmental Protection Agency, 186 p. (EPA/600/3-90/041; PB-90-212606).

Test Substance: Ethanedial

Remarks: Source, Purity of test substance: No information given

Method

Method/guideline followed*:* Standard procedure from US Federal Register (Vol. 50, No. 188; Part 797; Sec. 797.1050), with some modifications based on more recent EPA publications. Included in Appendix A of the reference.

Test type*:static*
GLP: Yes [ ] No [X]
Year *(study performed):*1989
Species/strain # and source: *Selanastrum capricornutum* derived from strain ATCC 22662 obtained from the American Type Culture Collection, Maryland, USA, which is maintained at the Environmental Research Laboratory – Corvallis.
Element basis*:* growth inhibition.
Exposure period: 4 days (96 hours)
Analytical monitoring: Cell counts made daily by an electronic particle counter. The cell counts were converted to mg/L dry weight for each flask and compared to the average dry weight of the three control flasks. This was multiplied by 100 to get percent growth relative to the control. Chemical analysis was not done, as they were not able to detect a standard of glyoxal with any of their available instrumentation.

Statistical methods: Median effect values and fiducial limits were derived by regression analysis using Statgraphics software. The regression curve that best fit the data was used, and the EC_{50} then calculated.

Test Conditions*:*

• *Test organisms*

♦ *Laboratory culture:* see above
♦ *Method of cultivation*: described in detail in the reference. Culture is maintained by daily consecutive aseptic transfer to 50 mL of liquid algal assay media.
♦ *Controls:*

• *Test Conditions*

♦ *Test temperature range:* Range was °C (± not indicated), rather than °C ± 2 °C
♦ *Growth/test medium chemistry (hardness, alkalinity, pH, TOC, TSS, dissolved oxygen, salinity, EDTA*): The test solution contained 300 ug/L EDTA, as this was found to be necessary to support sufficient algal growth, as confirmed by "recent ASTM proceedings".
- To test glyoxal, pH adjustment was necessary because of the tendency of glyoxal to cause a drop in pH below the tolerable range of the test organism. This was necessary for 1000 mg/L glyoxal. pH was measured at start and end of each assay. pH range was 7.1-7.2 for the low conc. (62.5 mg/L), and 9.3 (initial)-4.98 (final) for 1000 mg/L conc.

♦ *Dilution water source:* not described, purified by reverse osmosis (R.O)

♦ *Exposure vessel type: 125 mL plugged Erlenmeyer flasks. 5 concentrations x 3 replicates + 6 controls = 39 flasks. Flasks contain 50 mL test sample + diluent solution.*

♦ *Water chemistry in test (pH) in at least one replicate of each concentration (at start and end of the test):* pH measured at start and end of test only in highest and lowest concentration.

♦ *Stock solutions preparation (vehicle, solvent, concentrations):*

♦ *Light levels and quality during exposure:* light incubation range 360-440 foot candles.

• *Test design (number of replicates, concentrations): see above*
• *Method of calculating mean measured concentrations: NA*

Results

Nominal concentrations (mg/L): 62.5, 125, 250, 500, 1000
Measured concentrations (mg/L): NA
Element value:
EC_{50} (96 h) = 149 mg/L (nominal); 95% fiduciary limits: (0-350 mg/L)

Was control response satisfactory: Yes [X] No [ ] Unknown [ ]
Statistical results (as appropriate): Results plotted using Statgraphics program (test concentration yields, expressed as a percent of the control vs. test concentrations). Regression model with best fit chosen, and EC_{50} calculated, as well as 95% fiducial limits. In this case R^2= 82.3%

Remarks*:*

• *Biological observations*

♦ Cell density at each flask at each measuring point: yes
♦ Growth curves: Yes
♦ Percent biomass/growth rate inhibition per concentration: yes
♦ Observations:

Conclusions

Reliability: 2 **-** Satisfactory reliability

Remarks:
- Not OECD study but test procedure comparable to guidelines/ standards with acceptable restrictions;
- Study that has met basic scientific principles;
- The main deficiencies of the study are the lack of measured test concentrations, and lack of substance purity information.

Last changed: May 20, 2010

Appendix II: Upper bounding estimates of daily intake (µg/kg-bw per day) of ethanedial by the general population of Canada

Route of exposure	Estimated intake (µg/kg-bw per day) of ethanedial by various age groups							
	0–6 months[1]			0.5–4 years[4]	5–11 years[5]	12–19 years[6]	20–59 years[7]	60+ years[8]
	Breast milk fed[2]	Formula fed[3]	Not formula fed					
Ambient air[9]	3.71E-2			7.95E-2	6.20E-2	3.52E-2	3.03E-2	2.63E-2
Indoor air[10]	0.26			0.56	0.43	0.25	0.21	0.18
Drinking water[11]	0.00	8.71E-4	3.27E-4	3.7E-4	2.9E-4	5.50E-5	4.61E-5	4.54E-5
Food and beverages[12]	0.00	0.00	83.8	41.2	22.4	24.1	26.5	21.6
Soil[13]	4.76E-9			7.68E-9	2.5E-9	6.01E-10	5.04 -10	4.96E-10
Total intake	**0.30**	**0.30**	**84.1**	**41.8**	**22.8**	**24.4**	**26.7**	**21.9**

[1] Assumed to weigh 7.5 kg, to breathe 2.1 m^3 of air per day, to drink 0.8 L of water per day (formula fed) or 0.3 L/day (not formula fed) and to ingest 30 mg of soil per day (Health Canada 1998).

[2] No data were indentified on concentration of ethanedial in breast milk.

[3] For exclusively formula-fed infants, intake from water is that amount required to reconstitute formula. No data on ethanedial levels in formula were found; however, concentrations of ethanedial in drinking water were used in this model (see footnote 11). Approximately 50% of non-formula-fed infants are introduced to solid foods by 4 months of age and 90% by 6 months of age (NHW 1990).

[4] Assumed to weigh 15.5 kg, to breathe 9.3 m^3 of air per day, to drink 0.7 L of water per day and to ingest 100 mg of soil per day (Health Canada 1998).

[5] Assumed to weigh 31.0 kg, to breathe 14.5 m^3 of air per day, to drink 1.1 L of water per day and to ingest 65 mg of soil per day (Health Canada 1998).

[6] Assumed to weigh 59.4 kg, to breathe 15.8 m^3 of air per day, to drink 1.2 L of water per day and to ingest 30 mg of soil per day (Health Canada 1998).

[7] Assumed to weigh 70.9 kg, to breathe 16.2 m^3 of air per day, to drink 1.5 L of water per day and to ingest 30 mg of soil per day (Health Canada 1998).

[8] Assumed to weigh 72.0 kg, to breathe 14.3 m^3 of air per day, to drink 1.6 L of water per day and to ingest 30 mg of soil per day (Health Canada 1998).

[9] The mean concentration of ethanedial identified in ambient air in Ontario was 1.06 µg/m^3 from a daily monitoring site in 2000 (Aiello 2009).

[10] No data were identified for indoor air concentration in Canada. Indoor air concentrations were determined from a study in the United States; however the concentrations were at a lower concentration than ambient air, therefore Canadian ambient air data were used as a surrogate (see footnote 9). Canadians are assumed to spend 21 h indoors each day (Health Canada 1998).

[11] In the absence of monitoring data from Canada, a modelled estimate of an ethanedial concentration of 8.17×10^{-3} µg/L was used to calculate the upper-bounding estimates for drinking water exposure (ChemCAN 2003).

[12] No data were identified for the concentration of ethanedial in foods in Canada. Studies from Europe and Japan find concentration levels of ethanedial ranging from 0.9 – 1.7 mg/kg in food stuffs such as yogurt, honey, beer and wine (Arribas-Lorenzo 2010, Weigel 2004, Barros 1999, Yamaguchi 1994). Intake to beer and wine was removed from the intake estimate for children under the age of 11. Other food types were retained in the estimate calculation for non-formula-fed infants and children under the age of 11.

[13] In the absence of monitoring data from Canada, a modelled estimate of an ethanedial concentration of 1.19×10^{-3} µg/kg was used to calculate the upper-bounding estimates for soil exposure (ChemCAN 2003).

Appendix III: Upper-bounding estimates of exposure to ethanedial from consumer products

Consumer product	Assumptions	Exposure estimate
Paper	For standard multi-use paper, 500 sheets (dimensions 17 × 22 inches per sheet) weigh ~ 9 kg; therefore, one sheet weighs (9 kg)/500 = 0.018 kg. As a standard sheet of paper has dimensions of 8.5 × 11 inches, one standard sheet of paper weighs (0.018 kg) × [(8.5 × 11)/(17 × 22)] or ~0.0045 kg (~4.5 g). It was very conservatively assumed that ¼ of all of the ethanedial in a sheet of paper was ingested (~ 1 g of paper). The highest total concentration of ethanedial detected in paper coatings is 3.7 mg/g (Environment Canada 2010x). It was assumed that the ethanedial was completely absorbed. **Estimated oral intake:** Intake = [Concentration (w/w) of ethanedial in paper × weight of paper eaten] / body weight For children 0.5–4 years (Health Canada 1998): Intake = [(3.7 mg/g) × (1 g)] / 15.5 kg = 0.24 mg/kg-bw	Oral dose: 0.24 mg/kg-bw per event
Architectural paint	The inhalation scenario assumptions are based on a WPEM (2001) default scenario for water-borne latex paint. Both a layer of primer and paint were applied to the wall. The inhalation scenario assumptions: - maximum weight fraction: 0.009% w/w (CPCA 2010) - frequency of use: 1/yr - room volume: 22 m^3 (5% of total house volume) - air exchange rate: 0.45 changes/hr - amount of paint used: 6.4 L - body weight: 70.9 kg (Health Canada 1998) - inhalation rate: 27.5 m^3/day - air data calculated over 20 day exposure The dermal scenario assumptions are based on a ConsExpo default scenario for water-borne latex paint (RIVM 2007). The dermal scenario assumptions: - maximum weight fraction: 0.009% w/w (CPCA 2010) - frequency of use: 1/yr - exposed surface area: 0.33 m^2 (arms and hands) - contact rate: 30 mg/min - release duration: 7.2×10^3 - body weight: 70.9 kg (Health Canada 1998)	Acute inhalation dose: 1.1×10^{-4} mg/m^3 0.11 µg/m^3 Acute dermal dose: 4.57×10^{-3} mg/m^3 4.6 µg/m^3

Acne face wash	The scenario assumptions are based on a ConsExpo default scenario for Cleansing lotion/wash (RIVM 2007) The dermal scenario used uptake by diffusion: - maximum weight fraction: 0.001% w/w (LNHPD 2010) - frequency of use: 730/yr - exposed area: 565 cm^2 - applied amount: 2.5 g - uptake fraction:0.1 - body weight: 70.9 kg (Health Canada 1998)	Chronic dermal dose: 7.1×10^{-5} mg/kg-bw per day (0.071 µg/kg-bw per day)
Body lotion	The scenario assumptions are based on a ConsExpo default scenario for Body lotion (RIVM 2007) The dermal scenario used uptake by instant application: - maximum weight fraction: 0.1% w/w (CNS 2010) - frequency of use: 730/yr - exposed area: 16900 cm^2 - applied amount: 8 g - uptake fraction: 1 - body weight: 70.9 kg (Health Canada 1998)	Chronic dermal dose: 0.23 mg/kg-bw per day
Face moisturizer	The scenario assumptions are based on a ConsExpo default scenario for facial make-up (RIVM 2007) The dermal scenario used uptake by instant application: - maximum weight fraction: 0.1% w/w (CNS 2010) - frequency of use: 365/yr - exposed area: 565 cm^2 - applied amount: 0.8 g - uptake fraction: 1 - body weight: 70.9 kg (Health Canada 1998)	Chronic dermal dose: 0.013 mg/kg-bw per day (13 µg/kg-bw per day)
Spray-on leave-in hair conditioner	The scenario assumptions are based on a ConsExpo default scenario for hair spray (RIVM 2007). Maximum weight fraction: 3% w/w (CNS 2010); frequency of use: 1/yr The inhalation scenario assumptions: - room volume: 10 m^3 - air exchange rate: 2 changes/hr - spray duration: 0.24 min - cloud volume: 0.0625 m^3 - inhalation rate: 27.5 m^3/day - uptake fraction: 1 - body weight: 70.9 kg (Health Canada 1998) The dermal scenario used uptake by instant application: - frequency of use: 104/yr - exposed area: 580 cm^2 (Health Canada 1995) - applied amount: 0.6g - uptake fraction: 1	Inhalation mean event concentration: 0.041 mg/m^3 Inhalation chronic dose: 0.021 µg/kg-bw per day Chronic dermal dose: 0.072 mg/kg-bw per day

	- body weight: 70.9 kg (Health Canada 1998)	
Shaving preparation	The scenario assumptions are based on a ConsExpo default scenario for delapiltory cream (RIVM 2007) The dermal scenario used uptake by instant application: - maximum weight fraction: 0.1% w/w (CNS 2010) - frequency of use: 17/yr - exposed area: 5820 cm^2 (Health Canada 1995) - applied amount: 5.5 g - uptake fraction: 1 - body weight: 70.9 kg (Health Canada 1998)	Chronic dermal dose: 0.0036 mg/kg-bw per day (3.6 µg/kg-bw per day)
Hair dye	The scenario assumptions are based on a ConsExpo default scenario for hair dye (RIVM 2007) The dermal scenario used uptake by instant application: - maximum weight fraction: 0.1% w/w (CNS 2010) - frequency of use: 10/yr - exposed area: 580 cm^2 (Health Canada 1995) - applied amount: 100 g - uptake fraction: 1 - retention factor of 10% was applied[1] - body weight: 70.9 kg (Health Canada 1998)	Acute dermal dose: 0.14 mg/kg-bw per event
Manicure preparation	The scenario assumptions are based on a ConsExpo default scenario for nail polish (RIVM 2007) The inhalation scenario assumptions: - maximum weight fraction: 1% w/w (CNS 2010) - frequency of use: 156/yr - room volume: 20 m^3 - air exchange rate: 1 changes/hr - applied amount: 0.25 g - release duration: 12 hr - inhalation rate: 36.7 m^3/day - body weight: 70.9 kg (Health Canada 1998) The dermal scenario assumptions: - exposed surface area: 4 cm^2 - applied amount: 0.05 g - body weight: 70.9 kg (Health Canada 1998)	Mean event concentration: 4.21×10^{-4} mg/m^3 (0.42 µg/m^3) Acute Inhalation: 7.6×10^{-7} mg/kg-bw per event Acute dermal dose: 7.1×10^{-3} mg/kg-bw (7.1 µg/kg-bw) per event

[1] Retention factor was applied for rinse-off products (2006 Cosmetics Exposure Workbook, New Substances Assessment and Control Bureau, Health Canada).

Appendix IV: Summary of health effects information for CASRN 107-22-2: Ethanedial (Glyoxal)[1]

Endpoint	Lowest effect levels[2]/Results
Laboratory animals and *in vitro*	
Acute toxicity	**Lowest Oral LD_{50}** (rat) = 640-770 mg/kg-bw (Societe Française Hoechst 1980). **Lowest Inhalation (aerosol) LC_{50}** (rat) = 2410 mg/m^3 (4 hr aerosol exposure) (Hoescht 1984a). **LOEC Inhalation (saturated atmosphere) (rat) =** 4200 mg/m^3 (converted from 44.13 g of test substance over 7 hrs with a air exchange rate of 600 L/h) (Hoescht Celanese Corp 1984). **Lowest Dermal LD_{50}** (rat) >2000 mg/kg-bw (BASF 1985a). Main effects: gastric irritation and kidney damage when administered orally. Inhalation of aerosols results in respiratory irritation. Inhalation of vapours in a near saturated atmosphere (4200 mg/m^3) resulted in increased breathing rate (Hoescht Celanese Corp 1984). Numerous other studies cited in: OECD SIDS 2003; WHO 2004
Short-term repeated-dose toxicity (≤ 30 days)	**LOEL (oral) =** 120 mg/kg-bw/day based on decreased body weight gain and decreased food intake. Rats (CrlCD(SD)BR) (6/sex/group) were exposed to 40, 120, or 400 mg/kg-bw/day (ethanedial converted from 40% glyoxal) via the drinking water for 28 days according to OECD guideline 407. Body weight gain was significantly reduced in the high-exposure group and slightly reduced in the mid-exposure group, both of which coincided with a decrease in food intake. Water consumption was decreased in males and females in a dose dependant manner beginning at either the lowest group or intermediate group, respectively. No exposure-related effects were seen on haematological, biochemical, or urinary parameters. No exposure-related macroscopic or histopathological organ changes were noted during or after autopsy (Societe Française Hoechst 1987) **LOEC (inhalation)** = 2.3 mg/m^3 based on local effects in the larynx. Groups of 5 male and 5 female Wistar rats were exposed to nominal concentrations of 0, 0.4, 2, or 10 mg/m^3 (Avg measured conc. = 0.6, 2.3, or 8.9 mg/m^3), with mean aerodynamic mass diameters of 0.8-1.2 µm, 6 h/day, 6 days/week for a 29 days (20 exposures total, nose-only). Animals of the mid- and high-exposure groups had minimal squamous metaplasia of the epiglottal epithelium in the larynx that was

Endpoint	Lowest effect levels[2]/Results
	accompanied by submucosal lymphoid cell infiltration. No other adverse effects were observed regarding any parameter (Hoechst 1995). **LOEL (dermal)** = 105 mg/kg-bw/day. C3H/HeJ mice were exposed daily for ten days to 25 µl of an 1:4 or an 1:8 dilution of either European glyoxal 40, or Aerotex 40 (both 40% commercial solutions of ethanedial), which correspond to 53, and 105 mg/kg-bw/day, respectively (converted using Health Canada reference values for mouse body weight). Exposure to the highest dose induced open sores on the skin. No other ill-effects were observed at either dose (Bushy Run 1982). No other oral, inhalation or dermal studies.
Subchronic toxicity (30 days ≤ 1 year)	**LOEL (oral)** = 107 mg/kg-bw/day based on decreased total protein levels in serum. Male Sprague-Dawley (SD) rats (5/group) were exposed orally via drinking water for 30, 60, or 90 days. Exposures were 188, 407, or 451 mg/kg-bw/day in the 30 day group, 135, 239, or 344 mg/kg-bw/day in the 60 day group, and 107, 234, or 315 mg/kg-bw/day in the 90 day group. Test substance was 98.7% pure ethanedial. At the end of each time period clinical and biochemical analyses were performed. In the 90-day study a dose-dependent decrease in food and water consumption was observed with significance being reached in the mid- and high-dose groups. Only water consumption was significantly reduced in the low-dose group. Dose – dependent decreases observed in the absolute weights of the liver, kidneys, and spleen were observed in all animals of all dose groups and exposure periods but were significant in the mid- and high-dose groups only. Decreases in: alanine aminotransferase (ALT) (30, 60, 90 day)), aspartate aminotransferase (AST) (30, 60 day), lactate dehydrogenase (LDH) (90 day – high dose only), albumin (30, 60, 90 day), and total protein (30, 60, 90 day) were observed in the mid- and high-dose groups. ALT was also decreased at the low dose in both the 30 and 60 day studies while total protein was also decreased at the low dose in the 60 and 90 day studies. Significant reductions in the activities of glyoxalase I and II were observed after 30 days (measured in liver and erythrocytes of the mid- and high-dose animals). No effects were seen on glyoxalase I or II in the longer exposure groups. Glutathione and 2-thiobarbituaric acid-active substance levels were examined and found to be unchanged in the liver, kidney, and erythrocytes. (Ueno et al. 1991a).

Endpoint	Lowest effect levels[2]/Results
	Following the study design outlined above, male SD rats (5-7/group) were exposed, via drinking water, to 315 mg/kg-bw/day or 298 mg/kg-bw/day for 90 or 180 days, respectively. At 180 days, body weight gain was reduced compared to controls. Absolute organ weights (liver, kidneys, spleen, heart) were reduced compared to controls. Relative weights of the liver, kidneys, and heart were increased in comparison to controls. Alanine and aspartate aminotransferase and lactate dehydrogenase levels were slightly reduced after 180 days. Total serum protein content was decreased significantly in the test substance groups. After 180 days polyps and haemmorrhage were observed in the forestomachs of 2 exposed rats (not considered treatment-related). At 90 and 180 days, 4 animals had slight swelling of the papillary epithelial cells was observed in the kidneys along with papillary edema and congestion of the lymph nodes located in the proximity of the kidneys (Ueno et al. 1991a) Wistar rats (10/sex/group) were exposed to 40% ethanedial in the feed, which converted to 32, 63, 125, or 250 mg/kg-bw/day ethanedial, for 90-days. High-dose males had significant, reversible decreases in body weight gain after 2 weeks. During this period food intake did not decline significantly. Absolute liver and kidney weights were significantly increased in the high-dose groups. No other organs were examined. No macroscopic or micropathological affects were observed in thoracic or abdominal organs. Biochemistry and hematology were not performed (Mellon Institute 1966). Fischer 344 rats (10/sex/group) were exposed to 0, 1000, 2000, 4000, 8000, or 16000 mg/L in the drinking water (converted to 0, 143, 286, 571, 1142, 2286 mg/kg-bw/day using HC reference values (Health Canada 1994). Study was designed to determine dose ranges for a chronic study. All high-dose animals were in a moribund state and were sacrificed after 12 days. Body and organ weights decreased significantly in a dose-related manner as did food and water consumption. The maximum tolerated dose (MTD) was identified in this study as 500 – 2000 mg/L (71 – 284 mg/kg-bw/day) for males, and 1000 – 4000 mg/L (142 – 568 mg/kg-bw/day) for females (NTP 1991a). B6C3F1 mice (10/sex/group) were exposed to 0, 1000, 2000, 4000, 8000, or 16000 mg/L in the drinking water (converted to 0, 200, 400, 800, 1600, 3200 mg/kg-

Endpoint	Lowest effect levels[2]/Results
	bw/day using HC reference values (Health Canada 1994)) for 90-days. All animals survived until study completion. Decreased body and organ weights were observed (7-30% bw in groups receiving ≥4000 mg/L). A dose-dependent reduction in food and water intake was also observed. No haematological or biochemical tests were conducted. Male mice of all dosage groups displayed histopathological changes within the salivary glands, which were described as secretion depletion. Effects were suggested to be due to poor palatability of the test substance at all concentrations (NTP 1991b). **LOEC (Inhalation)** = No studies identified. **LOEL (Dermal)** = No studies identified.
Chronic toxicity/ carcinogenicity (>1 year)	No oral chronic/carcinogenicity studies identified. No inhalation chronic/carcinogenicity studies identified. **Dermal chronic/carcinogenicity** Two groups of 40 male C3H/HeJ mice were exposed to different commercial formulations of ethanedial. Exposure was to 25 µl of an 1:8 dilution of either European glyoxal 40 or Aerotex 40 (40% ethanedial) applied topically 3 times/week throughout life (converted to 23 mg/kg-bw/day[a]. .No increased incidence of skin tumours was observed. Irritation and necrotic areas were evident on some exposed mice. Survival rates were increased in the exposure group compared to controls. A fibrosarcoma was observed in one exposed mouse, but was considered to be unrelated to exposure as this type of tumour was said to be frequent in this strain in this laboratory (Bushy Run 1982). **Tumour promotion study** 28 Male Wistar rats were treated for 8 weeks with *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG) in their drinking water (100 mg/L) along with a 10% sodium chloride supplement. Ethanedial was then administered at a concentration of 0.5% (approximately 907 mg/kg-bw/day) in the water for 32 weeks (week 8 to 40). As controls, 8 rats received only ethanedial and 30 rats received only MNNG. Adenocarcinomas of the stomach were significantly ($p>0.05$) increased in rats receiving ethanedial and MNNG (12/28) vs. rats receiving only MNNG (5/30). No tumours were observed in the rats receiving only glyoxal (0/8) (Takahashi et al.

Endpoint	Lowest effect levels[2]/Results
	1989). **Tumour initiation study** 20 CD-1 mice per group were exposed to 0.1 ml of 40% ethanedial (approximately 485 mg/kg-bw/day) on shaven dorsal skin twice weekly for 5 weeks. Positive controls received 0.1 ml 7,12-Dimethylbenz[a]anthracene (DMBA) twice weekly. Negative controls received 0.1 ml DMSO twice weekly. One week after the last initiation treatment mice were exposed to 2.5 µg 12-0-tetradecanoylphorbol-13-acetate (TPA) in 0.1 ml acetone or 0.1 ml acetone alone, twice weekly, for 47 weeks. No significant increase in skin tumour formation was observed (Miyakawa et al. 1991).
Developmental toxicity	**Oral Developmental NOEL (rat) ≥** 125 mg/kg-bw/day. 19-24 female Wistar rats were exposed to 40% ethanedial in water at the following levels: 0, 5, 25, or 125 mg/kg-bw/day (converted from 40% ethanedial). Exposure was between days 6-19 post-coitum. No developmental effects were observed at any level. Maternal toxicity was observed at the high-dose only – significantly reduced food consumption, significantly lower relative body weight gain (BASF & Clariant 2001). In a range-finding study, Sprague-Dawley rats were exposed by gavage to 0, 200, 800, 1200, 1600, or 2000 mg ethanedial trimeric dihydrate/kg-bw/day (0, 166, 663, 994, 1326, 1657 mg/kg-bw/day ethanedial) during gestation days 6-15. Decreased maternal weight gain was observed at 166 mg/kg-bw/day and above. Clinical signs of toxicity and decreased gravid uterine weight were observed at exposures greater than 663 mg/kg-bw/day and maternal deaths were observed at 994 mg/kg-bw/day and above (NTP 1991c). In the follow up to the above study, SD rats were exposed, by gavage, to 50, 150, or 300 mg ethanedial trimeric dihydrate/kg-bw/day (41, 124, 249 mg/kg-bw/day ethanedial) during gestation days 6-15. No maternal toxicity was observed at any dose in this study, nor were there any embryotoxic effects (NTP 1994a,b). New Zealand white rabbits were exposed by gavage to 0, 166, 331 mg/kg-bw/day ethanedial (converted from 0, 200, 400 mg/kg-bw/day ethanedial trimeric

Endpoint	Lowest effect levels[2]/Results
	dihydrate) on days 6-19 of gestation. At the high-dose, maternal toxicity and embryotoxicity were reported. Systemic toxicity was evident along with decreased weight and decreased foetal weight. The rapporteur discussed exposures of ≥200 mg/kg-bw/day as problematic due to the corrosive nature of the substance, which led to damage of the gastric mucosa in pregnant rabbits (NTP 1991d, 1992). A follow up study in rabbit administered 50 mg ethanedial trimeric dihydrate/kg-bw/day, which corresponds to 41 mg/kg-bw/day ethanedial, on days 6-19 of gestation. Maternal toxicity did not occur, except for minimal reductions in body weight gain and food consumption (NTP 1993).
Reproductive toxicity	No studies available.
Genotoxicity and related endpoints: *in vivo*	**Gene Mutation** **Negative:** *Drosophila melanogaster* sex-linked recessive lethal-mutation test, dominant lethal test, and reciprocal translocation (Barnett & Munoz 1989). **Micronucleus Formation** **Negative:** Swiss mice (5/sex/duration) exposed orally to 1000 mg/kg-bw (40% ethanedial) and sacrificed at 24, 48, or 72 h after exposure Polychromatic erythrocytes (bone marrow) were examined (Societe Francaise 1986b). **Chromosomal Aberration** **Positive:** Rats were exposed subcutaneously to 0.5 or 1 ml of 10% ethanedial. Animals were sacrificed 24, 48, or 72 h after exposure. Increased numbers of chromosome aberrations were observed in the duodenum, testes, and spleen. Liver and pancreas did not have increased chromosomal aberrations (Thomas 1958). **DNA damage** **Positive for unscheduled DNA synthesis (UDS):** Male F344 rats administered 120, 240, 360 or 400 mg/kg-bw orally. Sacrifice was 2 h after exposure. Positive in the pyloric mucosa (Furihata et al. 1985; Furihata & Matsushima 1989). **Negative for UDS:** Male Wistar rats exposed to 100, 500, or 1000 mg/kg-bw (40% ethanedial) orally. Sacrifice was 2 or 16 h after administration. Primary hepatocytes

Endpoint	Lowest effect levels[2]/Results
	were examined for evidence of UDS (CCR 1992). **Positive for DNA single-strand breaks:** Sprague-Dawley rats and F344 rats were exposed orally to 120, 240, 360 or 400 mg/kg-bw, and 5, 50, 500, or 550 mg/kg-bw, respectively. In both instances sacrifice was 2 h after exposure. In the SD rats a dose-dependent response was observed in the liver. A weak positive was observed in the spleen. In the F344 rats, a clear dose-dependent response was recorded in the DNA of the pyloric mucosa (Ueno et al. 1991b; Furihata & Matsushima 1989b).
Genotoxicity and related endpoints: *in vitro*	**Gene Mutation** **Positive for bacterial reverse mutation:** Multiple Ames' assays (plate incorporation or pre-incubation) performed with and without metabolic activation using *Salmonella typhimurium* tester strains: TA100, TA1535 (with metabolic activation only), TA102, TA2638 (without metabolic activation only), TA98, TA7006, TA104 (Sasaki & Endo 1978; Bjeldanes & Chew 1979; Oesch 1979; Levin et al. 1982; Suwa et al. 1982; Garst et al. 1983; Niemand et al. 1983; Yamaguchi & Nakagawa 1983; Hoechst 1984b; Marnett et al. 1985; Sayato et al 1987; Hoechst 1988; Shane et al. 1988; Aeschbacher et al. 1989; Kato et al. 1989; Ueno et al. 1991c; Dorado et al. 1992; Murata-Kamiya et al. 1997a). **Positive for bacterial reverse mutation:** *Escherichia coli* WP2uvrA/pKM101 with and without activation (Kato et al. 1989). **Positive for bacterial forward mutation:** L-arabinose-resistance test using *Salmonella typhimurinum* tester strains BA9 and BA13 without metabolic activation (Ruiz-rubio et al. 1985; Ariza et al. 1988). **Positive for mammalian cell forward mutation:** Mouse lymphoma test using L5178TK$^{+/-}$ cells, without metabolic activation (Wangenheim & Bolcsfoldi 1988). **Positive for mammalian cell reverse mutation:** Reversion of Chinese hamster ovarian AUX b1/GAT cells to adenosine-thymidine-glycine prototrophy. No data on whether an activation system was used or not (Taylor & Wu 1980; Taylor et al. 1983). **Positive for increased mutation frequency:** COS-7 cells carrying the pMY 189 plasmid (Murata-Kamiya 1997b). **Negative for mammalian cell forward mutation:** CHO-S/HPRT and V79/HGPRT tests (Taylor et al. 1983; Societe Francaise Hoechst 1986a).

Endpoint	Lowest effect levels[2]/Results
	Chromosomal Aberrations **Positive:** Chinese hamster ovary cells with and without activation (Henkel 1986). **Positive:** Chinese hamster V79 cells without activation. Not tested with activation (Nishi et al. 1989). **Sister Chromatid Exchange** **Positive:** Chinese hamster ovarian AUXB1 cells, CHO cells (normal), human lymphocytes (American Cyanamid Co. 1982; Tucker et al. 1989). **DNA Damage** **Positive unscheduled DNA synthesis:** Syrian hamster TC-SV-40 cells without metabolic activation (Cornago et al. 1989). **Positive for DNA single-strand breaks:** Mouse lymphoma L5178TK$^{+/-}$ cells (without metabolic activation) examined by alkaline-unwinding assay. Rat hepatocytes (with metabolic activation) examined by alkaline elution (Garberg et al. 1988; Ueno et al. 1991b). **Positive for DNA damage in bacteria:** *umu*, SOS chromotest, Rec assay, and DNA repair tests using *Salmonella typhimurium* TA1535/pSK1002, *E. coli* PQ37, *B. subtilis* H17 or M45, and *E. coli* K12 343/636 or K12 343/591, respectively. All tests were performed with and without activation except the *umu* test, which was performed with activation only. (Von der Hude et al. 1988; Matsui et al., 1989; Ono et al. 1991a,b; Hellmer & Bolcsfoldi 1992a). **Positive for Comet Assay in Mammalian cells** : Rat heapatocytes incubated with 0.5, 5, or 10 mg/ml ethanedial produced an increased tail moment in the comet assay at the lowest dose only. Authors suggested that extensive cross-linking at the higher doses prevented DNA migration, and therefore, no increase in tail-moment was observed at these higher doses. Using lower doses comet assays demonstrated DNA damage in TK6 cells (Kuchenmeister et al. 1998; Henderson et al. 1998). **Negative in host-mediated assay of bacterial DNA damage:** Intravenous injection of *E. coli* K12 343/636 or K12 343/591 into NMRI mice. Mice were exposed orally to 570 or 1700 mg/kg-bw (Hellmer & Bolcsfoldi 1992b). **Negative for DNA cross-links:** Rat hepatocytes (with metabolic activation) examined by alkaline elution assay (Ueno et al. 1991b). **Genome Damage**

Endpoint	Lowest effect levels[2]/Results
	Positive for endoreduplication: Chinese hamster ovarian AUXB1 cells without metabolic activation (Tucker et al. 1989). **Cell Transformation** **Negative:** Concentrations ranging from 1.65 µg/ml to 49.53 µg/ml [converted from reported values in µl/ml using a density of 1.27 g/cm^3 for ethanedial – exposures may be lower as it was not clear whether reported exposures accounted for the dilution (40% ethanedial) of the starting material] did not induce cell transformation in the C3H/10T1/2CL8 mouse embryonic cell line (Mason 1980a, b, c).
Irritation/sensitization	**Skin Irritation** Three female white Vienna rabbits were exposed topically (OECD guideline 404) to 0.5 ml Ethanedial 40 under semi-occlusive conditions. Exposure was for 4 hrs, after which the application patch was removed and the exposed area was washed with a 1:1 mixture of water and Lutrol. Observation was continued for 72 hrs and application sites were scored 30-60 min after removal, and again at 24, 48, and 72 hrs. No irritation was observed, erythema and oedema were scored as 0 in all animals (BASF AG 1985b). Patch tests were performed on the shaven backs of white rabbits. 30 or 40 % solutions of ethanedial were used. Exposure was for 1, 5, 15, minutes or 20 h. Additionally, rabbit ears were exposed for 20 h. Skin was washed with PEG 400 and then with 50% PEG 400 after each of the exposure times except the 20 h exposure. No or mild erythema was noted after exposures of 1 or 5 min, but a slight yellowing was noted 24 h after the end of exposure. The 15 min exposure induced mild oedema and scaling of the skin, scab formation and superficial necrosis were observed within 8 days. The 20 h ear exposure caused erythema, inflammation, and "minor skin defects" within 24 h of the end of exposure, and scab formation and slight necrosis within 8 days. Both 30 and 40 % pure ethanedial and a 40% solution of "raw" ethanedial caused similar effects (BASF AG 1963a,b). Adult white rabbits were exposed to 40% ethanedial solution topically onto a 5x7 cm shaven area on the back (no further protocol details given). After 3 days, a strong inflammatory response with erythema was noted. Necrosis and tissue

Endpoint	Lowest effect levels[2]/Results
	demarcation followed. Changes had disappeared almost completely 30 d after exposure. Histology confirmed that severe necrotic skin changes were occurring by the 4th day, but were less pronounced by the 9th day. By the 18th day, regeneration of the epidermis was observed (Ito, 1963). 10 µl of 29.2% ethanedial was applied to the depilated abdomen of rabbit(s) (number not provided). Slight irritation was indicated by minor hyperaemia. Irritation was rated a 2 on a scale out of 10 (Smyth et al. 1962). Occlusive application of 1.57 ml 40% ethanedial (798 mg/kg-bw) to the shaven skin of 5 Wistar rats/sex for a period of 24 h caused erythema in all exposed animals (BASF AG 1985b). **Mucous Membrane Irritation** White Vienna rabbits (1 male, 2 females) were exposed to 0.1 ml of Ethanedial 40 by instillation in to one eye each (OECD guideline 405). No washing was performed. Readings were performed at 1, 24, 48, 72 h, and at 8 days. Untreated eyes were used as controls. Conjunctival erythema and chemosis was observed at 1, 24, and 72 h after exposure and was considered slight to moderate. OECD grading was used to score effects. Mean scores were 0.0, 0.0, 1.6, and 0.8 for corneal opacity (max = 0), iritis (max = 0), conjunctival swelling (max = 2). All effects had cleared after 8 days. Ethanedial 40% was considered to be slightly irritating to the eyes of rabbits (BASF AG 1985c). Rabbits exposed to ethanedial 40% had developed reversible reddening and chemosis of the conjunctiva within 8 days (OECD guideline 405 study) (BASF AG 1963a,b). Ethanedial 30 or 40% was instilled into the conjunctival sac in rabbit. A mild to strong reddening was observed with mild oedema, inflammation, and clouding for the cornea. Effects disappeared within 1 to 2 weeks (BASF AG 1963a,b). A study was performed to compare the effects of pure ethanedial 40% and raw ethanedial 40% on rabbit eyes. Instillation of 0.05 ml into the conjunctival sac induced reddening, and severe inflammation of the conjunctiva. Pure ethanedial

Endpoint	Lowest effect levels[2]/Results
	caused hazy corneal clouding. Raw ethanedial caused a milky clouding of the cornea and scarification of the upper eyelid. Effects were reduced but still slightly visible after 8 days (WHO 2004). **Skin Sensitization** Maximization test: Twenty female Pirbright-White guinea pigs were injected with 0.1 ml of 20% ethanedial in the shoulder region. One week later, 300 mg of a 40% solution was applied epicutaneously and covered. Challenge was performed by occlusive epicutaneous application of 150 mg of a 10% solution 19 and 26 days after the intradermal injection. One death occurred after intradermal injection. Intradermal induction of the test substance in Freund's adjuvant/distilled water (1:1) induced necrotic changes and oedema. The first challenge caused slight erythema and distinct erythema in 1/19, and 6/19 animals, respectively. The second challenge caused a positive skin response in 11/19 animals, with 7/19 and 4/19 having slight or distinct erythema, respectively. Controls (10 animals) had no skin reactions at any time point. Ethanedial was considered sensitizing in this test (BASF AG 1987). Buehler test: Hartley guinea pigs (15 – 8males, 7 females/group) were administered induction exposures of 1.25, 5, and 20 %, occluded for 6 h, 3 times/wk, for 3 weeks. Two weeks after the last induction exposure, each animal in each group received challenge doses of 0.01, 0.03, 0.1, 0.3, and 1.0%. One week after the first challenge, each animal in each group received challenge doses of 0.3, 1.0, and 3.0%. One animal from each group induced with ethanedial died for reasons unknown. Primary irritation and cumulative irritation was dose-dependent and evident in all groups receiving ethanedial. Challenge with 1.0 and 3.0% elicited some response in all groups. The number of responses and the degree of response indicated that sensitization was dependent on dose received in the induction, and challenges. Ethanedial was considered a dermal sensitizer in this test (American Cyanamid Co. 1988).
Humans	
Immunotoxicity	Maximization test: 24 volunteers were exposed to a patch soaked with 1 ml of a 10% solution of ethanedial, 5 times for 48 h each. Applications were occlusive. Twenty-four hours after the final induction application, volunteers were exposed to

Endpoint	Lowest effect levels[2]/Results
	a 2% solution for 48-hours under occlusive conditions. The induction phase elicited slight irritation. Challenge exposures induced reactions in 24/24 volunteers. Ethanedial was considered sensitizing under the conditions of this study (Kligman 1966). In a repeated insult patch test, 24 men and 31 women volunteers were induced by applying "neat" material (Ethanedial – white powder) on a patch. 15 applications were received. Each application was for 24 h, followed by a 24 h recovery period. Applications were 3 times per week for 5 weeks. After a 14-day rest period a challenge patch was applied and left for 24 h under occlusive conditions. Examinations were made 24 and 48 h after the end of the challenge. No sensitization, fatiguing, or primary irritation was observed in any volunteer (Monsanto Co. 1969), 9/14 workers with known contact to 40% ethanedial had contact dermatitis localized predominantly to the lower arms and fingers. Patch testing with 20% solution elicited a positive reaction from 7 of 9 workers. Glucose tolerance tests were performed in all 14 workers and were negative (Ito 1963).
Other studies	Cerebrospinal fluid (CSF) samples from 6 patients with Alzheimer's disease (AD) and from 6 healthy controls were analyzed for ethanedial and methylglyoxal. No significant increase in the amount of ethanedial in the CSF was observed. The authors also performed a semiquantitative determination of glyoxalase-I positive neurons in the cerebral cortexes of 5 AD patients and 5 health controls.

1 Test substance concentrations have been converted from those reported to Ethanedial equivalents where necessary. All test concentrations refer to pure ethanedial.

2 LC_{50}, median lethal concentration; LD_{50}, median lethal dose; LOEC, lowest-observed-effect concentration; LOEL, lowest-observed-effect level.

Appendix V: Summary of (Q)SAR results for Ethanedial

Carcinogenicity

CAS RN	DEREK[1]	Oncologic[2]	CASETOX[3]				TOPKAT[4]			
	Cancer	Cancer	m-rat	f-rat	m-mice	f-mice	NTP m-rat	NTP f-rat	NTP m-mouse	NTP f-mouse
107-22-2 Ethanedial	P	P	ND	ND	ND	ND	P	IC	N	IC

Genotoxicity

CAS RN	Ames			ChrAb	Micronuclei Induction	Mouse Lymphoma mutation
	Derek	CT	TK	CT[#]	CT	CT
107-22-2 Ethanedial	P	ND	P[§]	P	ND	ND

CAS RN, Chemical Abstracts Registry Number, # - *in vitro* test (in cultured CHO cells); * - disodium component not modelled. [§] - Compound in database, not modelled.
ChrAb – Chromosomal aberration; m-rat – male rat; f-rat – female rat; CT – Casetox; TK - TOPKAT
ND – not in domain of model; IC – inconclusive; P – positive; N- negative

[1] [DEREK] - Deductive Estimation of Risk from Existing Knowledge [Prediction module on CD ROM]. 2008. Version 10.0.2. Cambridge (MA): Harvard University, LHASA Group. [cited 2009 Sep 30]. Available from: http://www.lhasalimited.org/index.php?cat=2&sub_cat=2# [restricted access].

[2][OncoLogic] Woo Y, Lai DY, Argus MF, and Arcos JC. 1995. Development of structure-activity relationship rules for predicting carcinogenic potential of chemicals. Toxicol Lett 79:219-228.

[3][CASETOX] [Prediction module]. 2008. Version 2.0. Beachwood (OH): MultiCASE. [cited 2009 Sep 30]. Available from: http://www.multicase.com/products/prod03.htm [restricted access].

[4] [TOPKAT] TOxicity Prediction by Komputer Assisted Technology [Internet]. 2004. Version 6.2. San Diego (CA): Accelrys Software Inc. [cited 2009 Jan 7]. Available from: http://www.accelrys.com/products/topkat/index.html