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Ocular changes after intravitreal injection of methanol, formaldehyde, or formate in rabbits.

Hayasaka Y, Hayasaka S, Nagaki Y.

Source

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Abstract

We evaluated the effects of intravitreal injection of methanol, formaldehyde, or formate on rabbit eyes. One hundred microl of 1% methanol, 1% or 0.1% formaldehyde, or 1% formate was injected in the vitreous cavity of the right eyes of rabbits. The eyes were examined by biomicroscopy and ophthalmoscopy weekly. One month after injection, the eyes were enucleated and examined histologically. One week after treatment the animals that received 0.1% formaldehyde showed retinal vessel dilation, and the rabbits that received 1% formaldehyde showed mild posterior subcapsular cataract and retinal vessel dilation and haemorrhages. One month after treatment, the animals that received 0.1% or 1% formaldehyde developed mild posterior subcapsular cataract and retinal lesions. Animals that received 1% methanol or 1% formate showed nearly normal optical media and fundi. Histologically disorganized retina and optic nerve were seen in eyes that received 0.1% or 1% formaldehyde. Eyes that received 1% methanol or 1% formate appeared histologically normal. Our findings indicate that intravitreal injection of formaldehyde causes retinal and optic nerve damage, while methanol and formate are not or less toxic to ocular tissues.

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[here, two authors in service to NASA struggle to make sense of the Iowa gang deceits about formaldehyde and formate, leading us to a new reference from Japan, 2001...]

http://www.nap.edu/openbook.php?record_id=11778&page=300

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Appendix 8: Formate, pages 342-363

[References, pages 361-363

"Although reports from Finland (Liesivuori et al. 1987; Liesivuori and Savolainen 1991) and the United States (Martin-Amat et al. 1977, 1978; McMartin et al. 1977, 1980; Jacobsen et al. 1988; Johlin et al. 1989; Tephly 1991; Eells et al. 1996) implicate formate as the toxic agent in methanol poisoning, dissenting investigators from Japan (Hayasaka et al. 2001) assert that formaldehyde is the toxic agent in methanol-induced eye toxicity."

Hayasaka, Y., S. Hayasaka, and Y. Nagaki. 2001. Ocular changes after intravitreal injection of methanol, formaldehyde, or formate in rabbits. Pharmacol. Toxicol. 89(2):74-78.

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One month after treatment, the animals that received 0.1% or 1% formaldehyde developed mild posterior subcapsular cataract and retinal lesions.

Animals that received 1% methanol or 1% formate showed nearly normal optical media and fundi. Histologically disorganized retina and optic nerve were seen in eyes that received 0.1% or 1% formaldehyde.

Eyes that received 1% methanol or 1%formate appeared histologically normal.

Our findings indicate that intravitreal injection of formaldehyde causes retinal and optic nerve damage, whilemethanol and formate are not or less toxic to ocular tissues.

PMID:

11555323]

[quotes given after quotes from formaldehyde appendix]

Appendix 7: Formaldehyde, pages 300-341

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[click on right arrow for next page]

[References pages 335-341]

TABLE 7-1 Physical and Chemical Properties

Formula	HCHO
Synonym	methanal, formic aldehyde, methyl aldehyde

Distribution and Elimination

In rodents, Buss et al. (1964) observed that about 40% of radiolabeled formaldehyde that was administered orally (7 milligrams per kilogram [mg/kg] of body weight dose) was eliminated as carbon dioxide within 12 hours (h), while another estimated 10% was eliminated in urine and 1% in feces.

Incorporation into macromolecules was postulated by the authors to account for much of the remaining radiolabeled carbon.

Formaldehyde is not generally expected to be absorbed into the blood-stream and carried as an unmetabolized molecule to other organ systems.

Thus, distribution and excretion are not thought to be significant considerations with formaldehyde exposure and toxicity.

Instead, its rapid metabolism and reactivity suggest that it will either be

metabolized or it will exert its toxic effects locally at the point of exposure.

It is postulated that the toxicity of formaldehyde is evidenced when exposure is of sufficient magnitude that this detoxification mechanism for formaldehyde is saturated and the reactive formaldehyde molecule is allowed to exert its effects on local tissues and macromolecules (for example, proteins and DNA) (Heck and Casanova 1990).

Eells et al. (1981) reported on the death of a 41-year [y]-old woman following the intentional ingestion of formaldehyde (620 mg/kg).

The woman was cyanotic and severely hypotensive when admitted to the emergency room and died within 28 h of admission.

Observed toxicologic effects prior to death included renal failure, abdominal pains, and symptoms of metabolic acidosis.

TABLE 7-2 Toxicity Summary

Dose/Route	Exposure Duration	Species	Effects	Reference
Acute Exposures				
520 mg/kg/dm, oral	One event	Human, male (n = 1)	Death; decreased blood pressure; GI irritation; cardiac arrest; acidosis	Burkhart et al. 1990
620 mg/kg/d, oral	One event	Human, female (n = 1)	Death; decreased blood pressure; GI irritation; acidosis; loss of consciousness	Eells et al. 1981
230 mg/kg/d, oral	One event	Human, female (n = 1)	Ulceration of esophagus mucosa; GI irritation; tachycardia	

Genotoxicity

Formaldehyde is a very reactive molecule that can readily interact with proteins, DNA, RNA, and other critical macromolecules (formaldehyde has an electrophilic carbonyl group that has an affinity for nucleophilic sites on these molecules) (Feron et al. 1991).

With humans, positive genotoxic effects include

lymphocyte chromosomal aberrations (Chebotarev et al. 1986) and sister chromatid exchange (Yager et al. 1986), along with increases in micronuclei formation in cells of the nasal passage.

As discussed previously, genotoxicity at sites distant from the portal of entry is unlikely.

This conclusion is generally supported by available genotoxicity studies (Klingerman et al. 1984; Dallas 1992; ATSDR 1999; WHO 2002).

There has been a particular research focus placed on the significance of DNA-protein crosslinks.

These complexes between DNA bases and proteins bound by crosslinks are formed in response to formaldehyde exposure (Swenberg et al. 1980).

It is postulated that these crosslinks could result in mutations and/or chromosomal aberrations if not repaired prior to cell replication (Morgan 1997; Klaassen 2001) or that repair and DNA regeneration could promote cell proliferation (Heck and Casanova 1990).

This potential is heightened by the observation that these crosslinks are relatively stable and can be formed following formaldehyde exposures that are not cytotoxic (Merk and Speit 1998).

However, there is still some debate in the literature as to whether genotoxic expression through DNA-protein crosslinks is the exact mechanism of formaldehyde carcinogenesis.

ATSDR utilized the NOAEL from this study (25 mg/kg/d) and applied a UF of 10 for extrapolation from animals to humans and another factor of 10 for human variability (final intermediate duration MRL of 0.3 mg/kg/d).

Consistent with the EPA oral RfD, the chronic-duration ATSDR MRL for formaldehyde is based on the results from Til et al. (1989).

This 2-y drinking water study, which observed significant histopathologic changes in the rat forestomach and glandular stomach, has been described previously in this document.

In deriving the MRL, ATSDR used the NOAEL of 82 mg/kg/d and applied a combined UF of 100 (10 for human variability and 10 for extrapolation from animals to humans).

This resulted in a final chronic-duration MRL of 0.2 mg/kg/d.

Applying drinking water risk assessment assumptions for the general population (70 kg body weight, 2 L/d ingestion), these MRLs for intermediate- and chronic-duration exposure to formaldehyde would correspond to drinking water equivalent concentrations of 10 mg/L and 7 mg/L, respectively.

State Approaches

Most states do not derive their own toxicity factors. Instead, they typically have a hierarchy of recognized sources of toxicity information (for example, EPA RfD, ATSDR MRL) that are utilized.
These toxicity factors are applied in risk calculations that also incorporate exposure assumptions and policy decisions (for example, evaluating a child versus an adult receptor) in the derivation of drinking water standards, action levels, or health-based guidelines.
As can be seen from those examples provided in Table 7-3, state drinking water guidelines for formaldehyde can vary significantly (0.03-6 mg/L).

Some of this variation can be explained solely by the addition of relative source contribution terms (often a factor of 5), which lower the allowable concentration of formaldehyde in water to account for non-drinking water exposures.

California, which has an action level of 0.1 mg/L for formaldehyde in drinking water, used the Til et al. (1989) NOAEL

but applied an additional UF (as compared to EPA or ATSDR) of 10 to account for inadequacies in the toxicity database.
The action level is also reflective of a relative source contribution of 20% in drinking water (that is, lowering the action level by a factor of 5).
California raised this action level in 2000, having previously used an action level for formaldehyde of 0.03 mg/L.
These observations may explain the low drinking water guidelines listed for New Jersey (0.1 mg/L) and Maine (0.03 mg/L), because it is not uncommon for other states to replicate California's approaches in developing their own guidelines.

It should be noted that all state and federal agencies appeared to use the systemic (noncancer) toxicity of formaldehyde as the basis for decision making.

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http://www.nap.edu/openbook.php?record_id=11778&page=342

8

Formate

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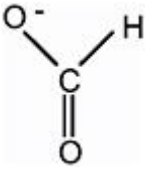
TABLE 8-1 Physical and Chemical Properties

Formula	$HCOO^-$
Chemical Name	Formate
Synonyms	Methanoate, carboxylate



TOXICITY SUMMARY

Formic acid is highly irritating and is caustic to the skin.

The main toxic effects associated with the ingestion of methanol (a metabolic precursor of formate) are metabolic acidosis and blindness from optic nerve injury.

The visual toxicity has been shown to be independent of pH (Martin-Amat et al. 1978).

The mechanism of cytotoxicity is believed to involve histotoxic hypoxia (the reduced ability of cells or tissue to accept and use oxygen from the blood, such as in cyanide poisoning) in aerobic cells caused by the impeding of oxygen metabolism (Liesivuori 1986).

Optic nerve cells have relatively few mitochondria and thus have a high sensitivity to histotoxic hypoxia (Jacobsen and McMartin 1986).

Formate acts as a reversible inhibitor of the mitochondrial cytochrome c oxidase complex (Nicholls 1975, 1976; Erecinska and Wilson 1980) with an apparent inhibition constant (*Ki*) between 1 and 30 millimolar (mM) (45-1,350 µg/mL) at pH 7.4, 30C (Nicholls 1975, 1976), depending on the reduction state of the system.

Inhibition of aerobic respiration is known to stimulate anaerobic glycolysis, resulting in increased lactate production and intra- and extracellular lactic acidosis (Seisjo 1992).

Metabolic and lactic acidosis are both hallmark features of severe human methanol intoxication (Erlanson et al. 1965; Koivusalo 1970;

Jacobsen and McMartin 1986).

The acidosis causes a decreased production of adenosine triphosphate (ATP) (Liesivuori and Savolainen 1991).

Interpretation of toxicity data from experiments involving exposures to methanol is complicated by interspecies as well as nutritional status-dependent differences in the rate of metabolism of methanol.

Although reports from Finland (Liesivuori et al. 1987; Liesivuori and Savolainen 1991) and the United States (Martin-Amat et al. 1977, 1978; McMartin et al. 1977, 1980; Jacobsen et al. 1988; Johlin et al. 1989; Tephly 1991; Eells et al. 1996) implicate formate as the toxic agent in methanol poisoning, dissenting investigators from Japan (Hayasaka et al. 2001) assert that formaldehyde is the toxic agent in methanol-induced eye toxicity.

No reports were found in the literature of ocular toxicity in humans who had ingested formic acid or formate, whereas ocular toxicity is commonly seen in cases of human exposure to methanol.

McMartin et al. (1980) reported severe metabolic acidosis and optic disc edema in two patients whose blood formate concentrations were 11.1 and 26.0 milliequivalents

(mEq) per milliliter when measured after hospitalization for methanol intoxication. The patient with the higher blood formate concentration died despite aggressive treatment (bicarbonate, iv 10% ethanol, and hemodialysis) of both patients.

Histotoxicity (manifested as extracellular calcium casts) has been reported in several tissues examined 1 h after the fifth daily dosing in male New Zealand rabbits (3,070 ± 220 g) given daily iv 1 mL doses of formate at 100 mg/kg (Liesivuori et al. 1987).

If we assume a body water volume to body mass ratio of about 700 mL water per kilogram, the rabbits had about 2,200 mL body water, and the formate concentration immediately after the injection would have been 307 mg/2,200 mL = 140 µg/mL body water.

Measured blood formate concentrations were 0.7 µmole/g (29 µg/mL) at 1 h after the fifth daily dose, 0.5 µmole/g (21 µg/mL) at 2 h, and 0.2 µmole/g (8.4 µg/mL) at 20 h.

Thus, it appears that 80% of the administered dose of formate had disappeared from the blood by 1 h postdosing, 5% more during the second hour postdosing, and 6% during the following 18 h.

This is consistent with a half-life of formate in rabbits of about 26 min.

The reduction of blood formate concentrations could be caused by either rapid sequestration of formate into other tissues or by rapid metabolism of the formate.

No mention was made in this report of any ocular toxicity in the rabbits.

In humans, permanent visual damage has been associated with prolonged exposures (>24 h) to blood formate concentrations > 7 mM (315 µg/mL) produced during methanol intoxications (Hayreh et al. 1977, 1980; Jacobsen and McMartin 1986).

The formate concentration in these patients (315 µg/mL) was higher than the initial concentration calculated for the rabbits (140 µg/mL) in Leisivuori's study.

Also, the fact that formate was being constantly produced by metabolism of ingested methanol means that elevated blood concentrations of formate were maintained for a prolonged period in methanol-intoxicated humans as opposed to rapidly declining to near background levels in the formate-treated rabbits, presumably from metabolism.

Clinical observations in methanol-intoxicated humans

have shown that in its initial stages, developing ocular toxicity can be reversed, even >24 h after methanol ingestion, by treatments such as bicarbonate, fomepizole or ethanol, iv folinic acid, or dialysis, which reduce blood formate concentrations and metabolic acidosis (Barceloux et al. 2002).

Such reversibility is consistent with a mechanism of formate toxicity involving gradual optic nerve histotoxicity due to prolonged metabolic hypoxia and with the reversibility of formate inhibition of cytochrome c oxidase (Nicholls 1975).

Martin-Amat et al. (1978) determined that the ocular toxicity (edema of the optic disc and loss of pupillary response) of methanol could be reproduced in male rhesus monkeys by iv infusion of sodium formate at 0.5 M, even when bicarbonate was administered to prevent acidosis.

Infusion at a rate of about 3.1 mEq/kg/h (formate at 140 mg/kg/h) after a loading dose of 1.25 mmole/kg (formate at 56 mg/kg) was calculated so as to maintain 10-30 mEq/L (450-1,350 µg/mL) formate in the blood over a period of 25-39 h.

Note that because a quasi-steady state was achieved, the infusion rate is also an estimate of the rate of removal of formate from the blood by metabolism and excretion.

In most of the treated animals, no pupillary response to light was observed at between 24 and 48 h after the onset of formate infusion, but in one of the four monkeys tested, normal pupillary reflexes and only moderate optic disc edema were observed at 25 h after initiation of treatment.

In this monkey, the maximum blood concentration of formate achieved was 540 µg/mL at 25 h postinitiation of treatment, compared to 900-1,530 µg/mL for the other three monkeys, as measured at later times (39-50 h post-initiation of treatment).

The onset of ocular toxicity generally occurred more rapidly in the formate-treated animals than in monkeys

that had received methanol in previous studies.

A more sensitive test for ocular toxicity (measurement of the reductions in the a and b waves of electroretinograms, [ERGs]) was used by Eells et al. (1996) to demonstrate that intraperitoneal (ip) injections of methanol in a regimen that maintained blood formate concentrations at 4-6 mM (180-270 µg/mL) for 60 h showed evidence of causing retinal dysfunction in the absence of retinal histopathology in nitrous oxide (N_2O)-treated rats.

The retinal dysfunction was not correlated with any clinical signs.

The rats in this experiment were made to more closely resemble humans and nonhuman primates in their methanol sensitivity by using subanesthetic concentrations of N_2O to selectively inhibit formate oxidation by inactivating the enzyme methionine synthetase, thereby reducing the production of tetrahydrofolate (Eells et al. 1996).

The 4-6 mM (180-270 µg/mL) concentrations of formate in blood were maintained in this experiment by ip injections of methanol at 2 g/kg in 12 h intervals.

Significant reductions in ERG a-wave and b-wave amplitude were not observed until 60 h after methanol administration, although blood formate concentrations reached the plateau level of about 180 µg/mL by 12 h, the earliest time of measurement.

Another group of rats in this experiment was treated with higher doses of methanol so that blood formate concentrations increased almost linearly from a baseline of 0.8 mM (36 µg/mL) to 7 mM (315 µg/mL) at 12 h to 15 mM (675 µg/mL) at 60 h after methanol administration.

ERGs of these high-dose rats showed reductions in the b wave as early as 24 h after methanol administration and profound attenuation or complete elimination of the b wave by 48-60 h after methanol administration.

Significant but less pronounced reductions in the a wave were seen in the high-dose animals.

Histopathologic changes including edema in the outer nuclear layer and vacuolization in the photoreceptors and the bases of retinal pigment epithelium cells were seen in the high-dose but not the low-dose rats at 48 h after methanol administration.

The ocular toxicities of 1% methanol, 0.1% or 1% formaldehyde, or 1% formate were compared in rabbits after a single intravitreal injection of 100 µL (Hayasaka et al. 2001).

The eyes were examined ophthalmoscopically at 1 d, 2 d, 1 week (wk), 2 wk, and 1 month (mo) after treatment.

Mild inflammation was seen at 1 d and 2 d for all chemicals, but by 1 wk, the eyes treated with methanol and formate appeared nearly normal while those treated with 0.1% formaldehyde showed retinal vessel dilation and those treated with 1% formaldehyde showed mild posterior subcapsular cataract, retinal vessel dilation, and retinal hemorrhages.

At 1 mo, animals who received 0.1% or 1% formaldehyde showed mild subcapsular cataract, vessel dilation, and juxtapapillary retinal hemorrhages.

Histopathologic study of the eyes showed nearly normal retinas in animals that received vehicle, methanol, or formate, but disorganized ganglion cell layer and outer nuclear layer in eyes that received 0.1% formaldehyde, and markedly disorganized retina in eyes that received 1% formaldehyde.

Similar differences were seen in the optic nerves, with formaldehyde-treated eyes showing vacuolizations.

The calculated intravitreal concentrations of the treatment chemicals were methanol at 700 µg/mL, formate at 700 µg/mL, and formaldehyde at 70 µg/mL and 700 µg/mL.

The report did not consider the potential for dilution of intravitreal chemicals into the systemic circulation.

Equally importantly, because these experiments involved a single dose of formate and methanol, the resulting reversible intracellular hypoxia may not have been prolonged enough to produce lasting injury, whereas the high reactivity of formaldehyde would be expected to cause immediate local injury.

From the above observations, it appears that the production of ocular toxicity by formate requires that the formate blood concentration remain elevated (≥180 µg/mL) for at least 24 h.

There are not sufficient data, however, to predict with confidence the effects of repeated low doses of formate

such that blood concentrations oscillate between toxic and nontoxic concentrations.

The amount of formate needed to achieve a blood formate concentration (180 µg/mL) in humans that has been reported (Eells et al. 1996) to cause electrophysiologic toxicity (but no histopathology) in retinas of N_2O-treated rats for exposures of up to 60 h would require ingestion of a bolus of formate at about 9.1 g.

This value was calculated (180 mg/L × 70 kg × 0.72 L/kg = 9,070 mg) assuming that formate is distributed throughout the water that makes up 72% (Lentner 1981) of a 70 kg body, assuming 100% uptake of ingested formate and no metabolism or excretion so that peak concentrations could be achieved and maintained.

Subchronic and Chronic Exposures (≥10 d)

No reports were found describing exposures to formate with durations of 10 d or more.

Genotoxicity

In tests by the National Toxicology Program (NTP), formic acid was found not to be mutagenic to *Salmonella typhimurium*, with or without metabolic activation (Thompson 1992). No genotoxicity studies on mammalian cells were found.

Reproductive Toxicity

No reports were found describing the reproductive toxicity of formate.

Developmental and Fetal Toxicity

No reports were found describing the developmental or fetal toxicity of formate.