HEALTH EFFECTS OF
PROJECT SHAD
CHEMICAL AGENT:

METHYL ACETOACETATE

[CAS# 105-45-3]

Prepared for the National Academies
by
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Health Effects of Methyl Acetoacetate
This report deals primarily with the biological health challenges engendered by the agent that is the subject of the report. Nevertheless, this report also incorporates, by reference and attachment, a supplement entitled "Psychogenic Effects of Perceived Exposure to Biochemical Warfare Agents".

The supplement addresses and describes a growing body of health effects research and interest centered upon the psychogenic sequelae of the stress experienced from perceived exposure to chemical and biological weaponry. Because awareness of exposure to agents in Project SHAD logically includes the exposed person possessing also a perception of exposure to biochemical warfare agents, the psychogenic health consequences of perceived exposure may be regarded as additional health effects arising from the exposure to Project SHAD agents. This reasoning may also apply to simulants and tracers.

Therefore, a general supplement has been created and submitted under this contract to address possible psychogenic effects of perceived exposure to biological and chemical weaponry.

Because such health effects are part of a recent and growing public concern, it is expected that the supplement may be revised and expanded over the course of this contract to reflect the actively evolving literature and interest in the issue.
SPECIAL NOTE ON CITATIONS AND AUTHORITIES

Citations to particular authorities shall be according to the name of the principal author, or in the case of references to an entire book, to the chief editor. The citation shall be appended outside the sentence or last in a series of sentences that cites the information or quotation from that authority. The year of publication will be appended to clarify the particular source cited from those several under the same principal author’s name. If the same author has two works published in the same year, the name of the publication in which it appears will be added for clarification. If it comes from a book, the name of the chief editor will be added.

All citations will be referred to authorities listed in the “Bibliography” section at the end of this report. If an author's name is cited in the text unambiguously, there will be no parenthetical listing of the source at the end of the sentence and the source in full will appear in the “Bibliography” section.
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I. EXECUTIVE SUMMARY

Methyl acetoacetate bears the chemical formula C5H8O3 (structured CH3COCH2COOCH3) and has a molecular weight of 116.11. Its Chemical Abstracts Service (CAS) registry number is 105-45-3. Its common alternative name is "Acetoacetic acid, methyl ester". Its density/specific gravity is 1.0762. At room temperature, the chemical is a colorless liquid with an agreeable odor. Its most common use is in the fragrance industry. Methyl acetoacetate was used as a simulant for sarin in at least two tests over the course of Project SHAD.

Methyl acetoacetate is generally regarded as being a mild to moderate irritant to the skin and mucous membrane, but with the capability (sometimes overlooked in secondary sources) of severe corrosive effect on the eye if directly contacted. The ocular exposure effect has been demonstrated in one older rabbit study. Secondary sources indicate gastrointestinal difficulties (nausea, vomiting, diarrhea) if it is swallowed, based upon the general characteristics of irritant toxic chemicals. Other effects extrapolated from general effects of irritant substances include swelling, redness and pain at any dermal site of exposure, but also particularly on mucous membranes. Mouth, nose, and eyes are especially susceptible. Irritants also commonly cause cough, tachypnea, and wheezing after inhalation exposure.

There do not appear to have been any published studies of chronic exposure. Recently, however, two Japanese research laboratories have examined methyl acetoacetate’s toxicity with greater thoroughness and a more updated focus on mutagenicity and carcinogenicity. They obtained results generally consistent with earlier studies on the questions of acute exposure. They also found no indication of mutagenicity or carcinogenicity. One mutagenicity test did yield a tentative finding of genotoxicity, but this was explained, after failure to replicate the effect in confirmation testing, to be the result of methyl acetoacetate’s alteration of the test medium’s pH. Methyl acetoacetate is also nowhere reported as a carcinogen.

One noticeable aspect of the literature on methyl acetoacetate has been the presence of significant discrepancies or omissions in the major secondary sources when compared with the primary studies or earlier secondary studies. These include 1) listing the wrong animal species used in a study, 2) providing dose figures not stated in the study being reported on, 3) offering a possibly misleading description of the animal lethality of one inhalation test, 4) omitting note of the substantial ocular toxicity of methyl acetoacetate, and 5) failing to update with later studies, including especially the recent Japanese laboratory studies. Issues of this type exist in the sources on methyl acetoacetate toxicity recommended by the Defense Department and extend to such standard or authoritative sources as Toxnet’s HSDB (Hazardous Substance Data Bank), Patty’s Toxicology, RTECS (during its existence as a publicly-owned resource), and the Merck Index.
II. BACKGROUND DATA

Physical Chemistry

Methyl acetoacetate bears the chemical formula C5H8O3. It is structured CH3COCH2COOCH3, and possesses a molecular weight of 116.11. Methyl acetoacetate’s CAS number is 105-45-3. Its most common alternative names are acetoacetic acid, methyl ester; and methylacetoacetate [single word]. Other alternative names include: acetoacetic acid, methyl ester; aceoacetic methyl ester; butanoic acid.; 3-oxo-, methyl ester, 1-methoxybutane-1,3-dione; methyl acetylacetonate; methyl acetylacetone; 3-oxobutanoic acid methyl ester. (HSDB) Project Shad literature has used the abbreviation MAA.

Its density/specific gravity is 1.0762. (HSDB) Methyl acetoacetate’s vapor density is 4.0 (where air = 1.0). The substance’s vapor pressure is 0.892 mmHg at 25 deg C. Its viscosity is 1.704 cPa at 20 degrees Celsius. (HSDB) At room temperature methyl acetoacetate is a colorless liquid with an agreeable odor. Its boiling point is about 171.7 degrees Celsius. Methyl acetoacetate is soluble in water (38g/100ml) and miscible with alcohol and ether. (HSDB)

Common Use

Methyl acetoacetate is commonly used in the fragrance industry. It is typically used as a solvent for cellulose esters, and usually synthesized by the reaction of methyl acetate with sodium methoxide. Eastman Kodak is a major manufacturer of methyl acetoacetate. (HSDB) Methyl acetoacetate has also been tested as a food for chicks, but found to be inefficient in terms of caloric absorption. (Opdyke) The chemical can also appear as a product of the degradation of the pesticide Mevinphos. (Erichson)
III. TOXICITY PROFILES

General

Studies on the toxicity of methyl acetoacetate are somewhat rare in the literature. Studies of chronic effects are absent. Enough has been done, however, to recognize a level of acute toxicity and incorporate it into standard industrial hygiene. The Association of American Railroads, for example, advises “avoid[ing] breathing [methyl acetoacetate] vapors…wear appropriate chemical protection gloves, boots, and goggles.” (HSDB) Hydrolysis and metabolic degradation take place quickly as methyl acetoacetate acid is a common human metabolite. (HSDB)

As the following discussion and subsections will elucidate, methyl acetoacetate is generally regarded as a mild (low to moderate) irritant. A rabbit (Draize) study in the 1940s has shown it to have a more intense corrosive effect directly on the eye. No mutagenic effects have been found in a bacterial test but a chromosomal aberration test showed genotoxic effects which were not replicated in a confirmation test, suggesting that methyl acetoacetate’s effect on the test medium’s pH was the cause of the genetic damage.

Labeled an “irritant” substance, methyl acetoacetate may have the following acute effects commonly observed with irritant materials (HSDB):

1) General -- Swelling, redness and pain at any site, especially at mucous membranes. The mouth, nose, and eyes are especially susceptible to these effects.
2) Inhalation exposure -- Cough, tachypnea, and wheezing are common.
3) Ingestion -- Nausea, vomiting and diarrhea are possible.
4) Dermal -- Redness, swelling and pain may occur.

Sinonasal neoplasms have been associated with inhalation of certain irritant materials like wood dust. (HSDB)

Below are results of published toxicology testing of methyl acetoacetate.

Acute Oral.

Source & Year: Smyth, 1948
Test: Range Finding Test
Result: LD$_{50}$ = 3 g/kg/day
Dose: Unspecified
Species: Rat

Source: Ohta, 2003
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Test: OECD Test Guideline 401
Result: LD$_{50}$ > 2g/kg [i.e. LD$_{50}$ exceeded maximum dose administered]
Dose/Duration: 0g/kg/day and 2g/kg
Species: Rat

**Acute Dermal**

Source: Smyth, 1948  
Test: Range Finding Test  
Result: LD$_{50}$ > 10 ml [no deaths from maximum dose]  
Dose/Duration: Four day poultice  
Species: Guinea pig

Source: Opdyke, 1978  
Result: Slightly Irritating  
Dose/Duration: "Full strength" 24 hr. under occlusion  
Species: Rabbit

Source: Opdyke, 1978  
Test: Sensitization  
Result: No Sensitization  
Dose/Duration: 8% concentration in petrolatum  
Species: Human

**Acute Ocular**

Source: Carpenter, 1946  
Test: Direct eye injection  
Result: Severe Toxicity (equivalent grade with other known causes of vision loss and corneal burns such as ethylene imine and ethylene chlorhydrin)  
Dose/Duration: .005ml (in 40% concentration)  
Species: Albino rabbit

(The MSDS (Material Safety Data Sheet) of the Aldrich Chemical Company warns unambiguously that methyl acetoacetate “can cause blindness”.) (Aldrich Chemical Company)

**Acute, Inhalation**

Source: Smyth, 1948  
Test: Range Finding Test  
Result: Maximum for No Deaths -- 8 hr.  
Dose/Duration: "Saturated vapors", 4 hour exposure, "series differing by a ratio of 2"
Species: Rat

**Subchronic Oral**

Source & Year: Ohta, 2003  
Test: OECD Combined Repeat Dose and Reproductive Developmental  
Result: NOEL > 1000 mg/kg/day [no effect found at any level]  
Dose/Duration: 0, 100, 300, 1000 mg/kg/day (Gavage)  
Species: Rat

**Reproductive/Developmental**

Source & Year: Ohta, 2003  
Test: OECD Combined Repeat Dose and Reproductive Developmental  
Result: NOEL > 1000 mg/kg/day [no effect]  
Dose/Duration: 0, 100, 300, 1000 mg/kg/day (Gavage)  
Species: Rat

(Pregnant rat exposure to another irritant material, N-methylpyrrolidone, resulted in offspring with impaired relative performance on high-level learning tests.) (HSBD)

**Mutagenicity**

No mutagenicity was found in 2003 bacterial testing, and none also was found in a pH-adjusted OECD Guideline 473 Non-Bacterial (Chinese hamster lung tissue) confirmation test following initial indications of chromosomal aberration.

Source & Year: Ohta, 2003  
Test: Bacterial Test -- Guidelines for Screening Toxicity Testing of Chemicals (Japan) & OECD Guidelines 471, 472  
Result: Not Mutagenic  
Dose/Duration: -S9 mix; 0, 313, 625, 1250, 2500, 5000 mg/plate  
Species: Salmonella typhimurium TA 100, TA 1535, TA 78, TA 1537, E. coli WP2 uvr A

Source & Year: Ohta, 2003  
Test: Non-Bacterial Test for Chromosomal Aberration, OECD Guideline 473 & Guidelines for Screening Toxicity Testing of Chemicals (Japan)  
Result: Induced Structural Chromosomal Aberrations and Polyploidy at 1.2 mg/mL on short-term test, but no chromosomal aberrations observed during pH-adjusted conditions in confirmation test. Suggested by investigators: Aberrations were caused by methyl acetoacetate lowering pH in medium
Dose/Duration: 0, 0.3, 0.6, 1.2 mg/mL (10mM, high concentration); short-term and continuous treatment, +S9 mix and -S9 mix; confirmation test with pH adjustment only short-term, 0 and 1.2 mg/mL, +S9 mix
Species: Chinese hamster (lung tissue)

**Carcinogenicity**

Methyl acetoacetate is also nowhere reported as a carcinogen. (HSDB, Bisesi)

(The Hazardous Substance Data Bank (HSDB) notes in general that inhalation of certain irritant materials (e.g. wood dust) has been associated with sinonasal neoplasms.)
IV. PSYCHOGENIC EFFECTS

Psychogenic effects specifically resulting from exposure to methyl acetoacetate are not reported in the literature. Psychogenic effects of perceived exposure to biological and chemical weaponry agents are treated in the supplement “Psychogenic Effects of Perceived Exposure to Biochemical Warfare Agents.”
V. SECONDARY LITERATURE ISSUES

Omissions or discrepancies can be noted in the secondary sources on the subject of methyl acetoacetate. Below is a detailed listing of many of these.

HSDB/Toxnet Monograph on Methyl Acetoacetate

1) There is no updated reference to the Japanese laboratory studies. (Ohta)
2) Methyl acetoacetate is described simply as a "low to moderate irritant to eyes" despite a finding of severe corrosive-type reaction in the eyes. (Carpenter)
3) The monograph states merely that an "8-hour exposure to saturated vapor was non-lethal to rats" while the original study as reported does not rule out lethality after a longer exposure.
4) Though noting the existence of the Opdyke monograph on methylacetoacetate under “Laboratory Methods”, the HSDB monograph does not appear to use the information and results of the studies cited in Opdyke’s Food and Cosmetics Toxicology article. (Opdyke)

Patty’s Toxicology

The standard reference’s methyl acetoacetate monograph section (Bisesi) contains the same omissions as HSDB above with an added misidentification of rabbit rather than guinea pig as test species in the 1948 Range Finding Test. (Smyth)

RTECS – Record of Toxic Effects of Chemical Substances (1993 Monograph on Methyl acetoacetate – a publicly produced monograph prior to 2001 transfer of RTECS to Elsevier)

1) It misidentifies rabbit as species in 1948 Range Finding Test. (Smyth).
2) Cites an Acute Oral LD$_{50}$ from the 1948 Range Finding Test of 3.228 g/kg where source merely states 3.0 g/kg. (Smyth)
3) Describes the dose in the Acute Eye test (Carpenter) as 2 mg. It appears that this is an application of specific gravity/density to primary source’s use of .0005ml and a 40% concentration (Carpenter).

SIRI -- Vermont Safety Information Resources, Inc. – MSDS on Methyl Acetoacetate

1) The MSDS lacks discussion of recent Japanese studies on methyl acetoacetate (Ohta)
2) Misidentifies species in Acute Dermal Range Finding Test as rabbit rather than guinea pig.
3) It also contains the discrepancies noted in RTECS--1993 public monograph

ATSDR – Agency for Toxic Substances and Diseases Registry

The online database contains no FAQ/monograph on methyl acetoacetate.
**Merck Index – Section on Methyl Acetoacetate**

For toxicity information, it refers the reader to the 1948 Range Finding Study (Smyth) rather than the far more comprehensive and more recent (1978) *Food and Cosmetics Toxicology* monograph on methyl acetoacetate. (Opdyke)

**PubMed/MEDLINE**

There is a complete absence of studies relating to methyl acetoacetate’s toxicity, despite the existence of a 1978 monograph in a key publication (Food and Cosmetics Toxicology) which includes clinical studies. (Opdyke)

**Online Defense Department Commentary on Methyl Acetoacetate’s use in Project Shad**

The Purple Sage/Project Shad website of the Department of Defense states of methyl acetoacetate that “potential health effects consist of low to moderate eye, skin, and respiratory tract irritation and possible gastrointestinal irritation with nausea, vomiting, and diarrhea. EPA does not consider methlyacetoacetate to be a hazardous material. It is not a known carcinogen.” (Special Assistant) That page also adds a set of recommended links to SIRI, and to a page at [http://www.hbcollege.com](http://www.hbcollege.com).

On the webpage specifically for DTC Test 69-31, Toxnet/HSDB is a recommended link as well. (Office of the Assistant)

1) The above description of effects does not note the more severe corrosive effect methyl acetoacetate can have on the eye.
2) Primary sources specifically reporting or detailing formal studies (animal or human) of gastrointestinal irritation have not been found.
3) The link to HSDB/Toxnet has the content deficiencies and discrepancies cited above for HSDB.
4) The link to SIRI has the content deficiencies and discrepancies cited above for SIRI.
5) The link to [http://www.hbcollege/chem/lab/organic/gilbert3e/resources/studenttools/dl/e_mmsds.pdf](http://www.hbcollege/chem/lab/organic/gilbert3e/resources/studenttools/dl/e_mmsds.pdf) is "dead", i.e. the site no longer available on the World Wide Web. Apparently the whole domain of www.hbcollege.com is also no longer available.
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