Spaceflight Maximum Allowable Concentrations for Ethyl Acetate

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INTRODUCTION: Ethyl acetate is a simple organic compound that occurs naturally and is used industrially as a solvent. It has been detected in the ISS atmosphere and is known to off-gas from building materials. As NASA astronauts have been and will be exposed to ethyl acetate during space missions, Spaceflight Maximum Allowable Concentrations (SMACs) were developed following an extensive review of the available literature.

METHODS: Toxicological data relevant to SMAC development was collected from electronic databases using principles of systematic review, and from previous assessments and reviews of ethyl acetate.

RESULTS: From an initial pool of over 35,000 studies, 10 were identified as studies appropriate to support SMAC development. The toxicological properties of ethyl acetate are relatively straightforward. Ethyl acetate is rapidly absorbed and converted by carboxyesterases to ethanol. At concentrations on the order of 400 ppm for 4–8 h, most volunteers experienced mild irritation but no lasting effects. In subchronic animal studies, mild sedative effects and changes in body weight and weight gain were observed at 750 ppm and above.

DISCUSSION: Numerous studies were identified to support the development of both short- and long-duration SMACs. No chronic studies were available, but the high quality of the subchronic studies and the short half-life of ethyl acetate support extrapolation to longer durations.

KEYWORDS: SMAC, spaceflight, International Space Station, astronaut, spaceflight environment, air quality, ethyl acetate, volatile organic compounds, offgassing.

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Ethyl acetate is a simple organic compound that occurs naturally in fruit and as a byproduct of fermentation (hence its presence in wine and other spirits).[35,40] It is "generally regarded as safe" (GRAS) by the United States Food and Drug Administration and is used as an approved flavoring agent in food and pharmaceuticals. Industrially, it is used as a solvent and is manufactured on a tremendous scale. Ethyl acetate is commonly used to isolate hydrophobic fractions of natural products for use in commercial and medicinal applications.[30,41]

Occupational exposure to ethyl acetate occurs in settings where lacquers, inks, adhesives, coatings, or solvents are used.[51] A number of studies have examined potential exposures in nail salons, along with acetone, acrylates, and other volatile organic compounds.[28] Numerous safety values are available for ethyl acetate (**Table I**).

Ethyl acetate is rarely flown as part of a payload to ISS, but it is occasionally detected in ISS air by the Air Quality Monitors (AQMs) and in routine sampling through mini grab sample containers (mGSCs). Ethyl acetate off-gasses from building materials[49] and has occasionally been detected at low levels in off-gas testing for NASA vehicles and equipment.[8]

METHODS

A strategy for gathering scientific data using principles of systematic review was designed according to the guidelines provided by the Office of Health Assessment and Translation and similar to that employed by the Agency for Toxic

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Table I. Existing Safety Limits for Ethyl Acetate.

ORGANIZATION	VALUE	PPM	mg · m^{-3}	DATE
EPA	*P*-RfC$_{subchronic}$	0.2	0.7	2013
	p-RfC$_{chronic}$	0.02	0.07	
OSHA	PEL	400	1470	
	STEL			
NIOSH	REL	400	1470	1992
ACGIH	TLV	400	1470	2001
CDC	IDLH	2000	7200	
SCOEL	8h TWA	200	730	2008
	STEL	400	1470	
MAK		400	1440	1958

Substances and Disease Registry in their toxicological profile for antimony.[2,39] A PECOT (population, exposure, comparators, outcomes, timescales) table was developed to clarify the criteria for inclusion in the review (**Table II**). Briefly, the systematic review sought to identify reliable and robust research studies in humans and laboratory animals which examine numerous toxicological endpoints following exposure to ethyl acetate and which identify explicit dose descriptors (e.g., NOAEC) that may serve as points of departure for SMAC development.

Table II. PECOT Parameters for Systematic Review for Ethyl Acetate Toxicity Data.

Populations	Humans
	Laboratory animals
Exposures	Inhalation
	Ingestion
	Dermal
	Other
Comparators	Controls
	Subjects exposed to lower doses
Outcomes	Eye irritation
	Skin irritation
	Skin sensitization
	Respiratory sensitization
	Systemic effects
	Respiratory
	Cardiovascular
	Gastrointestinal
	Hematological
	Musculoskeletal
	Hepatic
	Renal
	Endocrine
	Dermal
	Ocular
	Body weight
	Metabolic
	Other effects
	Immunological effects
	Neurological effects
	Reproductive effects
	Developmental effects*
	Cancer
Timescales	Acute
	Subacute
	Subchronic
	Chronic
	Other

* Developmental effects are not considered in setting SMACs, as they are not relevant to spaceflight exposure scenarios. However, data from these studies can be informative for other endpoints.

The final search term was: "ethyl acetate" OR "Acetic acid ethyl ester" OR "Acetic acid, ethyl ester" OR "Acetic ether" OR "Acetidin" OR "Acetoxyethane" OR "Ethyl ethanoate" OR "Ethyl ester" OR "Ethyl acetic ester" OR "141-78-6". Gathering of potential data sources was performed in October 2018. An additional search was conducted in August 2021 to verify that no additional studies had been published after the earlier review date. During the process of systematic literature review, numerous errors in dating of articles were noted in the results from the Toxline Database. Also, numerous references from HERO were not gathered in the search. Further exploration indicated that searching the HERO database via its web interface did not gather all resources even with the sole search term "ethyl acetate," though it appeared in the title of numerous resources cited in other documents and found on HERO through other search strategies.

Careful curation of the data sources was required, as several sources were duplicated by different authors; the root cause of this is the provenance of the documents through regulatory submissions. For example, studies conducted by Union Carbide[9] and Haskell Laboratories[11,12,14] were also identified as emanating from the trade association representatives who submitted the documents for regulatory review (i.e., CM Price and

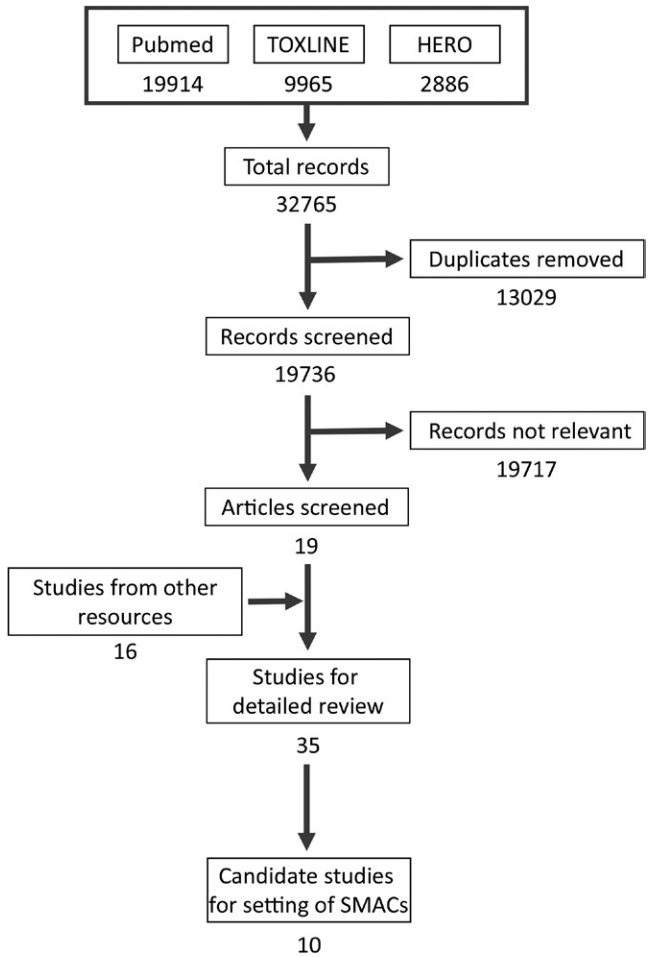

Fig. 1. Study selection process and metrics for systematic review of ethyl acetate toxicology.

LA Spurlock). From the electronic resources, 32,765 records were gathered. The use of ethyl acetate to extract natural product mixtures is responsible for the large number of initial resources identified by database searches. Screening reduced the original data set to 19 relevant articles and reports (**Fig. 1**).

To ensure our review was comprehensive, we scrutinized prior assessments of ethyl acetate which included a PPRTV,[21] a data summary generated by EPA's Integrated Risk Information System,[22] two occupational safety values from the EU,[23,24] a Cosmetic Ingredient Review (CIR),[29] and a SIDS Initial Assessment Report.[40] From review of summary sources, 16 studies were added for a total of 35 studies for detailed review. Several of these are overlapping or redundant, as numerous reports were generated from the same studies (**Table III**), and a subset of 10 studies were ultimately used for setting of SMACs. Each study was reviewed for Risk-of-Bias using the IRIS framework for assessing data quality. Of the 10 studies selected, only 1 (Nelson[36]) was regarded as "low confidence" based on a lack of available information for study design and interpretation. ACGIH, however, viewed this study as sufficiently robust to set their threshold limit value.[1,36] All other studies were rated as medium or high confidence.

RESULTS

Toxicokinetics

Ethyl acetate is rapidly absorbed during inhalation exposures in both animals and human volunteers.[37,50] The available data demonstrate ethyl acetate is also rapidly eliminated via enzymatic and nonenzymatic hydrolysis to ethanol and acetic acid.[16,29] Following ingestion exposures, the half-life of ethyl acetate in blood is on the order of 35 s and attributable primarily to rapid metabolism by carboxyesterases in organs.[18,26] In rats given intraperitoneal injections of ethyl acetate, high concentrations of ethanol were detected within 5 min, and ethyl acetate became undetectable after 20 min.[26] Ethanol predominated in the tissues of a 39-yr-old worker who died from acute ethyl acetate intoxication.[15]

In another study, rats were exposed to 500–10,000 ppm ethyl acetate via endotracheal tube. Accumulation of ethanol in rats only occurred in exposures exceeding 2000 ppm ethyl acetate.[23,26] At very high concentrations (e.g., 10,000 ppm), ethanol accumulates rapidly and causes respiratory depression. The European Commission's Scientific Committee on Occupational Exposure Limits (SCOEL) judged that, due to its

Table III. Summary of Relevant Toxicological Studies on Ethyl Acetate.

SPECIES AND NUMBER	EXPOSURE DURATION	TARGET EXPOSURE LEVELS (PPM)	RESULTS	DOSE DESCRIPTOR	LEVEL	REFERENCE
Human volunteers (4M, 4F)	2h × 6	15 ppm	No changes were observed in measures of eye or respiratory irritation.	NOAEC	15 ppm	20
Human volunteers (*N* = 10)	3-5m	200, 400 ppm	Subjects described 200 ppm as "objectionable" due to strong odor.	NOAEC LOAEC	200 ppm 400 ppm	36
Human volunteers (*N* = 16)	4 or 8 h	400 ppm	Increased reports of moderate irritation were reported among volunteers relative to controls.	LOAEC	400 ppm	45
Human volunteers (*N* = 32)		400 ppm	Increased reports of "annoyance" were reported among volunteers relative to controls.	LOAEC	400 ppm	46
Human volunteers (*N* = 24)	4h	400 ppm	Subjective reports of olfactory symptoms were markedly increased at 400 ppm.	LOAEC	400 ppm	32
Human volunteers (*N* = 4 and 6)	4h	200, 400 ppm	Irritation was not observed at 200 ppm, but mild irritation in eyes, nose, and throat were reported at 400 ppm in 2 of 6 subjects.	NOAEC LOAEC	200 ppm 400 ppm	34
CD Rat (14/sex/treatment)	6h	0, 600, 3000, 6000 ppm	No overt clinical signs were observed as a result of treatment. Dose-dependent changes in body weight were observed at all dose levels.	NOEL (neurotoxicity)	600 ppm	9
CFW mice (*N* = 8, male)	20m	0, 250, 500, 1000, 2000 ppm	Significant decreases in locomotor activity were observed in mice exposed to 2000 ppm but all were reversible after exposure concluded.	NOAEL (neurotoxicity)	1000 ppm	9
Rat	6h/day, 5d/week, 2 wk (60 h)	0, 1500, 3000, 6000 ppm	Decreased body weight and weight gains were noted in all exposure groups (only among females).	LOAEC	1500 ppm	9
Crl: CD BR rat	10 h over 2 wk	1500 ppm	Decreased body weight and weight gain were observed.	LOAEC	1500 ppm	11,12,13,14
Crl:CD BR rat (*N* = 40)	6 h/day, 5d/week, 89 d (385.5 h)	0, 350, 750, 1500 ppm	Microscopic lesions in olfactory tissues and minor reductions in weight gain in male rats were noted in 8 of 20 animals at 350 ppm.	NOEC*	350 ppm	11,12,13,14

*The study documents refer to this dose level as a LOAEC for body weight loss and nasal lesions in rats. EPA has determined that this dose level is a NOAEC as the body weight changes are not significant and the microscopic nasal lesions in rats are not relevant to human receptors.

rapid hydrolysis, ethyl acetate is unlikely to cause systemic effects and that the critical acute effect for ethyl acetate is irritation of the upper respiratory tract.[21]

According to Fleury and Wirth,[25] acute exposures to rabbits at 20,500 ppm (75,000 mg · m^{-3}) led to a reduction in blood pH of only 0.07; this indicates ethyl acetate exposures at concentrations that protect against irritation would not lead to acidosis.[29]

Crowell et al.[16] developed a PBPK model that incorporated the metabolic series approach to account for the sequential metabolism of ethyl acetate to ethanol and through subsequent steps. The model was populated using published data from in vitro and in vivo studies supplemented by findings from IV infusions of ethyl acetate in rats. Rats were given either an IV bolus of 10 or 100 mg · kg^{-1} ethyl acetate, or a 15-min infusion of 10 or 50 mg · kg^{-1}. Data from the 15-min infusion demonstrates a rapid decrease in blood levels of ethyl acetate while ethanol rises during the infusion and begins a slow decrease after the exposure ends. Similar conclusions can be drawn from the bolus dose. The predicted values are in very good agreement with data from the infusion studies. Additionally, the evidence demonstrates the elimination pathways for ethyl acetate (especially carboxyesterases) are not saturated at 100 mg · kg^{-1} as previously demonstrated.[16,18]

Toxicity

The most important toxicological outcomes following exposure to ethyl acetate vapors include irritation and neurological decrements. Acute exposures to higher concentrations can cause nausea and vomiting, and CNS depression. The odor threshold for ethyl acetate ranges from 3.6 – 245 ppm (24-900 mg · m^{-3}).[21]

Irritation

Several studies conducted in human volunteers have demonstrated ethyl acetate vapor can be irritating at high concentrations (> 400 ppm or 1470 mg · m^{-3}) and that 200 ppm (730 mg · m^{-3}) ethyl acetate carries an "objectionably strong" odor for unacclimated workers.[1,43] In a group of 10 volunteers exposed to ethyl acetate for 3–5 min, most reported 100 ppm (360 mg · m^{-3}) would be tolerable for an 8 h exposure and 200 ppm was not irritating but had an intense odor.[36] McCallum et al. reported irritation effects were not observed at 200 ppm for 4 h (N = 5), but were observed in two of six individuals at 400 ppm.[34] Kleinbeck et al. subjected 23 volunteers to ethyl acetate at 2 ppm, 400 ppm, and variable levels beginning at 5 ppm and peaking at 800 ppm (2900 mg · m^{-3}) four times during the exposure period of 4 h.[32] Half of respondents described the severity of olfactory symptoms as "rather much," "considerably," or "very, very much." Despite this result, the authors describe 800 ppm as "minimally irritating" and 400 ppm as "bearable during long-term exposure." Similarly, Seeber et al. exposed volunteers to 400 ppm ethyl acetate for 4–8 h and determined some irritation and annoyance occurs at that level.[45,46] Dwivedi et al. used 15 ppm ethyl acetate to mask the odor of acrolein during an irritation test for that substance, and no effects were observed from exposure to ethyl acetate at that level for 6 episodes of 2 h among 8 volunteers.[20]

Instillation of 1 drop of ethyl acetate into a rabbit eye led to reddening and slight conjunctival swelling that regressed after 1–2 d. In cats, concentrations higher than 4200 ppm (15,100 mg · m^{-3}) caused closed eyes and lacrimation.[25] Direct application of ethyl acetate to skin leads to defatting and damage to the strateum cornum.[29]

Acute Effects

The LC50 for ethyl acetate for rats is on the order of 55,500 ppm (200 g · m^{-3}) in rats and 12,500 ppm (45 g · m^{-3}) in mice.[5,44] Ethyl acetate was fatal in cats after a 15-min exposure to 43,000 ppm (155,000 mg · m^{-3}), while 9000 ppm (32,000 mg · m^{-3}) caused irritation and labored breathing. Exposure to 20,000 ppm for 45 min caused deep narcosis.[52]

Several summary sources reported the findings of Smyth and Smyth, in which three guinea pigs were exposed to ethyl acetate at 290 ppm (1030 mg · m^{-3}) in "gassing jars."[47] ACGIH noted the animals withstood ethyl acetate concentrations of 2000 ppm (7200 mg · m^{-3}) for 65 exposures (4-h each) without effects on body weights or clinical blood parameters.[1] The investigators observed anemia secondary to leukocytosis and liver damage in rabbits exposed to 4450 ppm (16,000 mg · m^{-3}) ethyl acetate.[1,47,52]

Bowen and Balster assessed the acute neurobehavioral effects of ethyl acetate on mice (N = 8) following a single 20-min inhalation exposure at 0, 500, 1000, or 2000 ppm (0, 1800, 3600, 7200 mg/m^3).[7] At the highest concentrations, ethyl acetate caused decreased locomotor activity and other behavioral changes. Spasmodic movements were observed at all concentrations tested, but these were not recorded in a robust way and thus cannot be evaluated. The animals recovered within minutes after removal from the exposure chamber.

DuPont de Nemours conducted a study in dogs to determine the comparative toxicity of three acetic acid esters: methyl acetate, ethyl acetate, and N-butyl acetate.[19] The exposure was to levels estimated to be approximately half of the dose required to induce narcosis; for ethyl acetate, this was 22 mg · L^{-1} (equivalent to 22,000 mg · m^{-3}, or 6100 ppm). The authors note the actual concentrations may vary as much as 10%. Two dogs per concentration were exposed for 40 min/d, twice a week, for 4 wk (total exposure time: 5.3 h). The measures used were generally subjective. Ethyl acetate was noted to elicit "excitement" in the dogs posttreatment, but not as potently as methyl acetate did. The symptoms were barking, whining, pawing, and walking with a staggering gate. One dog was noted to have tremor. Ethyl acetate induced vomiting, and moderately increased the rate of respiration (not quantified). Exposure to ethyl acetate was also said to induce "a trend toward circulatory abnormality" and a fall in venous blood pressure. Other effects included a rise in rectal temperature, irritation (salivation, lacrimation), and prolonged "unsteadiness." Given the nature of the experiments, this report is not informative in the setting of SMACs, but useful in terms of high-exposure effects.

Subacute Effects

Burleigh-Flayer et al. exposed groups of rats (10 males and 5 females per group) to ethyl acetate by whole-body inhalation at 1500, 3000, and 6000 ppm (5400, 11,000, and 22,000 mg · m^{-3}) for 6 h/d, 5 d/wk for 2 wk (i.e., a total of 60 h over 10 d).[9] Neurological symptoms were assessed via a functional observational battery (FOB) and motor activity testing before and after exposure. Body weights, clinical symptoms, and food and water consumption were reported through the exposure period. As reported by EPA, neurological symptoms were observed at 3000 and 6000 ppm, including decreased startle reflex, abnormal eye responses, and hypoactivity. Changes in body weight-corrected brain and ovary weights were noted in female rats at the upper concentrations. Concentration-dependent decreases in body weight and food consumption were also observed. A LOAEC of 1500 ppm was identified for this study, based on decreased food consumption. Human equivalent concentrations (HECs) were calculated using standard methodology but without the benefit of physiologically based pharmacokinetic modeling.

Subchronic Effects

The strongest body of evidence on ethyl acetate toxicity comes from a series of subchronic tests conducted at the Haskell Laboratory for Toxicology and Industrial Medicine. The investigators exposed Sprague-Dawley rats via chamber (i.e., inhalation) to 0, 350, 750, and 1500 ppm (0, 1300, 2700, and 5400 mg · m^{-3}) for 6 h/d, 5 d/wk for 13 wk (i.e., a total of 390 h over 65 d)[13] and examined neurotoxicological[12] and operant behavioral outcomes,[11] as well as olfactory pathology.[14] The top dose level was based on the subacute study conducted by Burleigh-Flayer et al.[9]

In the neurobehavioral study, 12–18 animals of each sex at each dose level were subjected to an FOB and motor activity assessment on nonexposure days or after a 4-wk recovery period. Diminished startle responses were observed in the 750 and 1500 ppm exposure groups. The investigators determined the decrement was a threshold effect related to frank narcosis seen at 5000–12,000 ppm in other studies. Changes in grip strength were observed in female rats at 350 and 1500 ppm but were determined not to be related to ethyl acetate treatment. No sensory or motor anomalies were identified via the FOB in this study. Neuropathological evaluation did not reveal any structural abnormalities.

No compound-related reductions in organ weight were observed, though spleen weight was lower and adrenal weight was higher in the highest treatment group. These changes were described as secondary to lower body weight. No compound-related effects were observed during gross pathology.[14] Microscopic pathological analysis was conducted on the neurological system, testes, and nasal mucosa. At the lowest dose level (350 ppm), microscopic lesions were observed in olfactory mucosa in 8 of 20 animals and were graded as minimal. These lesions were observed in 100% of animals at the higher dose levels and graded as "minimal to moderate" for the 750 ppm group and "minimal to severe" for the 1500 ppm group. As a result, no NOAEC could be established. 350 ppm might be considered as a LOAEC for rats in this experiment, on that basis. Lesions of this type are common in rats exposed to acetate esters due to tissue-specific liberation of acetic acid.[40] For that reason, and due to physiological and anatomical differences in nasal structures, their relevance to human health is uncertain.[10,27,40]

Concentration-dependent decreases in body weights were observed in both sexes, accompanied by decreases in food consumption. The EPA analyzed the changes in body weights and determined the reductions in body weight at the lowest dose level (350 ppm) was not physiologically significant. Thus, the NOAEC for ethyl acetate was determined to be 350 ppm, and a LOAEC at 750 ppm based on decreased body weights, food consumption, and startle responses. This study was chosen as the principal study for p-RfC by the EPA.[23] As noted by the EPA, data reporting was not sufficient to allow for benchmark dose modeling. As with the shorter-term study, HEC for each dose level were calculated using duration adjustment to a 98-d exposure period as no PBPK model existed at the time.

Crowell et al. applied their PBPK model to the data generated by Christoph et al. to generate human equivalent concentrations (HECs).[16] The model indicates that a dose level of 350 ppm in the study is commensurate with an HEC of 495 ppm for an 8 h/d, 5 d/wk (i.e., occupational) exposure and a continuous HEC of 119 ppm (based on blood levels of ethyl acetate). The authors describe the 350 ppm dose level as a LOAEC due to body weight losses, though EPA notes the "small decreases in body-weight gain and food efficiency at [350 ppm] are not considered biologically significant" and refers to this exposure level as a NOAEC.[23] Though the model is not validated with human toxicokinetic data for ethyl acetate, the ethanol portion of the model is considered robust given the wealth of data for that substance. However, the model did overpredict blood ethanol concentrations arising from whole-body ethyl acetate exposures in rats. The authors postulate this is due to lung-specific metabolism of ethyl acetate upon inhalation, whereas the PBPK model was calibrated using intravenous bolus doses. The HEC from this study will be considered as a candidate for the setting of SMACs with the addition of appropriate uncertainty factors. Given the calculated HEC for continuous exposure is threefold lower than the NOAEC, it is expected to produce similar SMAC values as use of a standard uncertainty factor of 3 for interspecies differences in toxicokinetics/toxicodynamics.

No controlled chronic inhalation studies are available for ethyl acetate. Limited data are available from numerous occupational cohorts exposed to ethyl acetate over longer periods, though generally other solvents are also present. ACGIH notes findings described by Patty in which workers were regularly exposed to 375–1500 ppm (1350-5400 mg · m^{-3}) for several months but showed "no unusual signs or symptoms"[1]. The Dutch Expert Committee for Occupational Standards reported workers who were exposed to ethyl acetate at concentrations ranging from 4200–14,000 ppm for 2 wk to several years suffered numerous symptoms of ongoing eye irritation

(lacrimation, edema on eyelids, conjunctival irritation).[29] Occupational studies of workers in paint spraying and a shoe factory are also discussed, but their confounding exposures to unspecified solvents and tolene/xylene make it difficult to determine whether any effects can be attributed to ethyl acetate.[29] As ethyl acetate is rapidly metabolized and eliminated, it is likely the duration of exposure is not a critical determinant of long-term toxicity (especially at lower concentrations).[29,42]

Mutagenicity/Genotoxicity

Ethyl acetate produces aneuploidy in yeast assays but does not appear mutagenic or genotoxic in the Ames, sister chromatid exchange, or chromosomal aberration assays.[4,31,53] When administered to mice interperitoneally (3 doses/week for 8 wk), no increase in tumors was noted, nor did one-time dermal application of ethyl acetate to the skin of mice produce any increase in papilloma incidence.[33,48] Basler also dosed hamsters with ethyl acetate via intraperitoneal injection (473 mg · kg^{-1}), and no increase in micronuclei was observed.[4] No chronic carcinogenicity studies are available for ingestion or inhalation exposures.

Existing Safety Values for Ethyl Acetate

ACGIH has set a Threshold Limit Value of 400 ppm (8-h time weighted average, TWA), based on Nelson et al. and includes the expectation that some workers may experience mild irritation.[1,36] This value is consistent with those endorsed by OSHA and NIOSH in the United States, and a maximum workplace concentration (MAK) set by Germany in 1958. The SCOEL re-evaluated their safety values on ethyl acetate in 2008 and promulgated an 8-h TWA value of 200 ppm and a Short-Term Exposure Limit (STEL; 15 min) of 400 ppm.[21] This value was predicated on the same data as ACGIH and supplemented by information from the subacute and subchronic studies in rats.

EPA's subchronic p-RfC of 0.2 ppm (0.7 mg · m^{-3}) was set based on the NOAEC of 350 ppm from subchronic studies in rats.[13] This level was adjusted to a HEC of 209 mg · m^{-3}, which was divided by 3000 to account for interspecies differences in toxicodynamics (3), interindividual differences in susceptibility (10), and lack of an acceptable two-generation reproductive or developmental toxicity study (10). The chronic p-RfC was divided by an additional uncertainty factor of 10 to address lifetime exposure from a subchronic study, rendering a final value of 0.02 ppm (0.07 mg · m^{-3}). As SMACs are set for healthy adults, they generally do not account for sensitive subpopulations as terrestrial safety values do. Also, given that SMACs are set for less-than-lifetime exposure durations in persons who are not pregnant (or likely to become pregnant), developmental toxicity is not considered.

Summary of Development of Updated SMACs

Spaceflight factors. When setting SMAC values, NASA occasionally includes an additional uncertainty/safety factor to protect against toxicological outcomes that may be compounded by exposure to the spaceflight environment. For example, hypercalcemia and hypercalcuria have been observed for all crew members as a result of weightlessness, and thus any chemical exposures impacting the remodeling of bone or modulation in circulating calcium levels might require an additional safety factor to reduce the hazard. However, the toxicological endpoints of interest for ethyl acetate (irritation in humans, body weight losses and slight neurobehavioral changes in animals) do not justify the use of an additional factor in setting SMACs.

EFSA's review of ethyl acetate mentions a study that suggests ethyl acetate may cause immunosuppression; however, this study involves an ethyl acetate extraction of latex from a plant in the Euphorbiacae family.[3,5] Administration of this extract caused reductions in T-cell and neutrophil counts. Given the wealth of evidence that humans experience altered immune responses in spaceflight, such a finding would be relevant to the setting of a SMAC. Unfortunately, given the ambiguous nature of the test substance, it cannot be determined whether ethyl acetate is responsible for those reductions. Leukocytosis was noted in rabbits exposed to 4450 ppm ethyl acetate in a prior study, but no such observations were made in animals exposed to lower concentrations.[47]

1-h and 24-h SMACs. Short duration SMACs (1-h and 24-h) apply to accidental releases or other emergency scenarios on a spacecraft, and as such the values are set to permit minor, reversible effects (such as mild irritation).

Data from ethyl acetate exposures in human volunteers suggests that 400 ppm (over 4 or 8 h) is mildly irritating.[32,34,45,46] These observations are the basis for ACGIH's TLV of 400 ppm (1440 mg · m^{-3}) with the notation that mild irritation may be expected in workers who are unaccustomed to ethyl acetate exposure.[1] Further, ACGIH relayed observations from Patty's Toxicology that "workers exposed regularly at concentrations from 375 to 1500 ppm for several months showed no unusual signs or symptoms"[1]. Therefore, the 1-h SMAC is set at 400 ppm. The available studies in human volunteers extend no longer than 8 h. Multiple studies indicate that 200 ppm is not irritating in 4- or 8-h studies, but many respondents listed it as having an objectionably strong odor. Thus, the 24-h SMAC is also set at 400 ppm (**Table IV**). This level may be associated with minor, reversible irritation and odor complaints but is consistent with SMACs for off-nominal scenarios.

7-d SMAC. Two studies are available to support a 7-d SMAC value: Burleigh Flayer et al.,[9] and Christoph et al.[13] The total exposure period for rats in the study conducted by Burleigh Flayer is 60 h, while the subchronic studies conducted by Christoph et al. exposed rats to ethyl acetate for 390 h (compared to 168 h in a 7-d period).[13] Further, the study conducted by Burleigh Flayer identified 1500 ppm as a LOAEC (based on decreased food consumption) while Christoph identified a NOEC of 350 ppm (decreases in body weight and food efficiency). The application of the 350 ppm NOAEC from Christoph divided by an interspecies uncertainty factor of 3 (with no adjustment for exposure duration) yields a 7-d SMAC value of 117 ppm (Table IV). Additionally, the direct application of the adjusted NOAEC for continuous exposures in humans posited by Crowell et al. suggests a 7-d SMAC of 119 ppm.[16] Although this is expected to

TABLE IV. *Spaceflight Maximum Allowable Concentrations for Ethyl Acetate.*

ENDPOINT	EXPOSURE CONCENTRATION (PPM)	SPECIES	UNCERTAINTY FACTORS					SPACEFLIGHT MAXIMUM ACCEPTABLE CONCENTRATIONS (PPM)					REFERENCE
			NOAEL	DURATION	SPECIES	SPACEFLIGHT	DATABASE	1h	24h	7d	30d	180d	1000d	
LOAEL irritation, 8h	400	Human	1	1	1	1	1	**400**	NA	–	–	–	–	32,34,45,46
LOAEL irritation, 8h	400	Human	1	1	1	1	1	NA	**400**	–	–	–	–	32,34,45,46
NOAEC body weight, 90d*	350	Rat	1	NA	3	1	1	NA	–	**117**	–	–	–	11,12,13,14
NOAEC body weight, 90d*	350	Rat	1	NA	3	1	1	NA	–	–	**117**	–	–	11,12,13,14
NOAEC body weight, 90d*	350	Rat	1	NA	3	1	1	–	–	–	–	**117**	–	11,12,13,14
NOAEC body weight, 90d*	350	Rat	1	NA	3	1	3	–	–	–	–	–	**39**	11,12,13,14

*The study documents refer to this dose level as a LOAEC for body weight loss and nasal lesions in rats. EPA has determined that this dose level is a NOAEC as the body weight changes are not significant and the microscopic nasal lesions in rats are not relevant to human receptors.

protect against both irritation and neurological effects, it is within the reported range of odor thresholds and may present a habitability concern even if not directly toxic.

30-d and 180-d SMACs. As ethyl acetate is rapidly metabolized and doesn't accumulate, the degree of toxicity is a function of exposure dose rather than duration. Irritation as a toxicological endpoint, resulting from exposure to substances with brief half-lives, is regarded as being concentration-dependent and not dependent on duration of exposure.[6,17,42] As a result, no duration extrapolation need be applied to determine SMAC levels for 30- or 180-d. Thus, the value of 117 ppm derived as described above will also serve for these values.

Development of Extended-Duration (1000-d) SMAC

Little data exists to support the development of a comparison value for 1000 d. Again, the toxicokinetic data appears to support the adoption of the 180-d SMAC for the 1000-d SMAC, as ethyl acetate is rapidly converted to ethanol, and the ethanol doesn't accumulate in laboratory animals until the exposure level exceeds 2000 ppm. For context, ethanol has been assigned a 1000-d SMAC of 1000 ppm (2000 mg · m^{-3}), though the level on ISS is more tightly regulated to avoid impacts to the water recovery system.

However, using data from a 90 d (390 h) study to establish a safety value for 1000 d (24,000 h) is not consistent with best practice, in the absence of supporting data. Thus, the applicable value for the shorter-term nominal SMACs will be divided by 3 to account for deficiencies in the available data. This results in a 1000-d SMAC of 39 ppm (140 mg · m^{-3}).

DISCUSSION

SMACs have been developed and adopted for ethanol.[38] The long-term SMACs were all set at 1000 ppm (1900 mg · m^{-3}) to protect against irritation of the eye and mucous membranes, along with flushing of skin and the possibility of hepatotoxicity. Hydrolysis of the acetate ester by carboxylesterases present in the nasal mucosa are likely responsible for the irritating effects of ethyl acetate at moderate concentrations (i.e., 400 ppm and greater).

One source of uncertainty is the designation of 350 ppm (from subchronic chamber exposures of rats to ethyl acetate) as a NOAEC in setting of SMACs for 30-, 180- and 1000-d durations. The investigator suggested 350 ppm is a LOEC in the context of lesions in olfactory tissue and in the context of body-weight gain and food efficiency in male rats.[13] With regard to the olfactory lesions, the anatomical differences between humans and rats and rats being obligate nose breathers complicate interpretation of the relevance to human health.[10,27] Also, these lesions were minimal at the 350 ppm dose level and limited to 8 of the 20 exposed animals (3 male, 5 female).

The reductions in body weight gain and food efficiency in male rats only were considered as not physiologically significant by EPA, though they were statistically significant.[23] Female

rats did not experience any significant decreases in body weight gain or food efficiency at the 350 ppm dose level, and the investigators note that this dose is a NOEC for female rats.[12]

Given the short half-life of ethyl acetate in vivo and the nature of the adverse health effects observed by the investigators, the application of this study to SMAC development generates SMAC values that are protective of astronaut health during long-term spaceflight.

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