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An Analysis of Human Response to the Irritancy of Acetone Vapors

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ABSTRACT: Studies on the irritative effects of acetone vapor in humans and experimental animals have revealed large differences in the lowest acetone concentration found to be irritative to the respiratory tract and eyes. This has brought on much confusion in the process of setting occupational exposure limits for acetone. A literature survey was carried out focusing on the differences in results between studies using subjective (neuro)behavioral methods (questionnaires) and studies using objective measurements to detect odor and irritation thresholds.

A critical review of published studies revealed that the odor detection threshold of acetone ranges from about 20 to about 400 ppm. Loss of sensitivity due to adaptation and/or habituation to acetone odor may occur, as was shown in studies comparing workers previously exposed to acetone with previously unexposed subjects. It further appeared that the sensory irritation threshold of acetone lies between 10,000 and 40,000 ppm. Thus, the threshold for sensory irritation is much higher than the odor detection limit, a conclusion that is supported by observations in anosmics, showing a ten times higher irritation threshold level than the odor threshold found in normosmics. The two-times higher sensory irritation threshold observed in acetone-exposed workers compared with previously nonexposed controls can apart from adaptation be ascribed to habituation. An evaluation of studies on subjectively reported irritation at acetone concentrations < 1000 ppm shows that perception of odor intensity, information bias, and exposure history (i.e., habituation) are confounding factors in the reporting of irritation thresholds and health symptoms.

In conclusion, subjective measures alone are inappropriate for establishing sensory irritation effects and sensory irritation threshold levels of odorants such as acetone. Clearly, the sensory irritation threshold of acetone should be based on objective measurements.

KEY WORDS: acetone, irritancy, irritation, (neuro)behavior, chemosensory aspects, adaptation, habituation.

I. INTRODUCTION

There is a wealth of information on the irritative effects of acetone vapor in humans and experimental animals. Various techniques have been used to study these effects of acetone, including symptom questionnaires and psychophysical measurements, and examinations of functional changes in both humans and laboratory animals. These studies reveal large differences in the lowest acetone exposure concentration found to be irritative to the respiratory tract and eyes, ranging from about 250 to 186,000 ppm.[1–24]

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This very wide concentration range may cause confusion in the process of setting occupational exposure limits for acetone.

Acetone, a colorless, highly volatile, flammable liquid with a mildly pungent odor is a high volume chemical (world-wide annual production: 3.9 million tons) that is used as an intermediate in the production of methacrylates, Bisphenol A, and other ketones, and as a solvent for different applications such as coatings, printing inks, adhesives, cleaning material, and in spinning and film casting processes. After inhalation exposure, acetone is rapidly absorbed via the respiratory tract of humans and laboratory animals. Acetone is uniformly distributed among nonadipose tissues and does not accumulate in adipose tissues and is rapidly cleared from the body by liver metabolism and excretion. The major excretion route is by exhalation of CO_2, and at higher doses as unchanged acetone, whereas urinary excretion is a minor route. Acetone can also be formed endogenously in the mammalian body from fatty acid oxidation.[25] Acetone is of a low order of acute inhalation toxicity (4-h LC50 in rats: 32,000 ppm[26]; RD50 in mice: 23,000 to 78,000 ppm.[27–29] Inhalation exposure of rats to 19,000 ppm for 8 weeks produced a reversible decrease in absolute brain weight.[30] No long-term inhalation studies have been found. Acetone is not considered to be genotoxic or mutagenic.[25]

Acute exposure of workers to acetone concentrations > 12,000 ppm for up to 4 h has been reported to produce unconsciousness, dizziness, unsteadiness, confusion, and headache.[31] Irritation of eyes, nose, and/or throat were reported at concentrations ranging from a few hundreds ppm (~250 to 1000 ppm),[1–18] to a few thousands ppm (~2500 to 8000 ppm),[19,20] and to several ten thousands ppm (~32,000 to 130,000 ppm).[21–24]

Humans dispose of two important nasal chemosensory systems. The free nerve endings of the trigeminal system innervate the walls of the nasal passages and respond to a large variety of volatile chemical substances. Human psychophysical studies indicate that stimulation of the trigeminal nerve contributes to a sensation of general nasal irritability provoked by (high) concentrations of the chemical. Olfactory receptors respond to these chemical stimuli usually at lower concentrations and with greater selectivity than do the trigeminal endings and are responsible for the discrimination of different odorous substances. In cases of total anosmia, the capacity to identify and to distinguish between odors is lost, while the response to nasal irritation is generally preserved.[32] Various experimental techniques can be used to study chemical-induced irritation, including symptom questionnaires and psychophysical measurements, and examinations of functional changes such as alterations in breathing frequency and pattern, bronchial and pulmonary function parameters, eye blinking frequency, and chemosensory evoked potentials, in both humans and laboratory animals.

The very wide range in acetone concentrations reported to be irritative to eyes, nose, and/or throat cannot be explained by differences in method sensitivity, inherent variability in biological response, or fluctuations in the acetone exposure concentrations. Acetone has a strong odor, and at least part of the wide variation in the findings may be due to insufficient distinction between olfactory and trigeminal stimulation in several of the studies. Moreover, to distinguish between olfaction and irritation may be difficult, because odor perception is subjective and can be misinterpreted as an irritancy response. Finally, adaptation and habituation to acetone vapor may play a significant role in the human response to this chemical.

The article aims to clarify the odor and sensory irritation thresholds of acetone vapor. It reviews the key studies on (sensory) irritation to understand the differences in methodologies used and explain the variations in the results.

II. METHODS FOR ASSESSING CHEMOSENSORY EFFECTS

Chemosensory stimulation of the nasal passages can be odorous (olfactory stimulation) or irritating (trigeminal stimulation) or both. Both effects are sensory phenomena that can be observed with psychophysical methods and that over a broad range of stimulation do not lead to cell or tissue damage. In this way they serve as warning signals to the presence of the chemical. To cite Engen:[33] An unknown odor is a warning signal to which one does not have an appropriate response; it causes uneasy arousal, but odor per se does not make one sick.

With further excessive chemical stimulation reflex mechanisms may be invoked. According to Alarie,[34] airborne chemicals capable of stimulating nerve endings in the respiratory tract can be classified as follows:

- Sensory irritant. A sensory irritant is a substance that when inhaled via the nose stimulates trigeminal nerve endings, evoke a burning sensation of the nasal passages, and inhibit respiration from that site. Coughing might also be induced by laryngeal stimulation.
- Pulmonary irritant. A pulmonary irritant is a chemical that when inhaled stimulates sensory receptors within the lung and increases respiratory rate with a decreasing tidal volume, resulting in a rapid shallow breathing. Its action as opposed to that of sensory irritants is to evoke a sensation of dyspnoea and breathlessness rather than an obvious painful sensation.
- Bronchoconstrictor agents. A bronchoconstrictor is a chemical that when inhaled induces an increase in resistance to air flow within the airways of the lung. The action can be via a direct effect on smooth muscles of the conducting airways, by a neural reflex, or by liberation of mediators such as histamine.
- Respiratory irritant. A respiratory irritant is a chemical that when inhaled can act as a sensory irritant, bronchoconstrictor, and pulmonary irritant. These chemicals are capable of all three actions, and there is little difference between the concentrations at which they are sensory irritant and pulmonary irritant.

In the literature, a clear-cut distinction between odor and irritation is not always made. Nevertheless, they can be distinguished on the basis of their characteristics. With olfactory stimulation, strong effects such as adaptation, a decrease in sensitivity to a prolonged odor stimulus is shown, whereas with irritancy, temporal summation is noticed. In general, the longer such an irritating stimulus lasts, the stronger the sensation. Weak levels of irritation may grow in sensory magnitude over periods as long as hours, whereas, in contrast, strong levels may grow over just seconds or minutes and then decline.[35] This effect will be counteracted in time by the effect of habituation, which is a decrease in responsiveness to stimulation by a chemical as a result of a repeated exposure over intervals long enough to restore sensitivity from adaptation, viz. one may come to tolerate or even like an initially unpleasant stimulus from mere exposure.[36] In reports on chemosensory effects of chemicals habituation is often confused with adaptation. Habituation can be considered as an unconscious coping behavior and is, like annoyance, subject to cognitive influences such as expectation or information.[37]

Airway irritation has been defined in many ways and many of the definitions found in the literature are directly related to the methods of measurement techniques with which it was assessed. Basically, three approaches to the measurement of irritation can be distinguished:

1. Behavioral effects. Well-being, mood states, and health complaints are stud-

ied by means of self-administered questionnaires or structured interviews that measure annoyance.[1-3, 11-18, 24, 38] This can be called *psychological irritation.*

2. Sensory effects. Often odor detection thresholds (olfactory stimulation) are considered an important warning signal to avoid adverse effects at higher exposure concentrations.[1, 2, 24, 37, 39-48] However, in general, odor detection (mal-odor) as such is not regarded as a toxicologically relevant endpoint. For odorous respiratory irritants, it may be problematic to distinguish between olfactory stimulation and *sensory irritation* (reflexes that follow trigeminal stimulation like nasal pungency and watery eyes). Up until now four methods are available to make such a distinction:

   - Use of anosmics along with normosmics.[21, 23, 49-52] The use of anosmics allows for determination of sensory irritation in the absence of odor-induced responses.
   - Use of a lateralization technique [24, 50, 53-54]
   - Measuring eye irritancy along with olfactory stimulation[23]
   - Recording of chemosensory evoked activity in the brain.[55-57] Various methods exist such as Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET), EEG Event-Related Potential Mapping (ERP) and Magneto-Encephalography (MEG). A pressing technical issue is how to best develop and use the various methods to obtain spatially detailed functional maps of the brain with information on functional connectivity. Methods based on MRI and PET are limited in terms of time resolution, whereas ERP and MEG, having excellent time resolution, have poor sampling characteristics and often uncertain spatial localizing power. At this moment, knowledge of the functional architecture of the human brain can best be obtained by combining a number of these non- invasive techniques. As such corti- cal areas activated by trigeminal stimuli can be clearly separated from areas activated by olfactori stimuli.[53,56,58-59]

Although it is possible to distinguish between olfactory and trigeminal stimulation, contradictory results have been reported.[54,60-61] On the one hand, trigeminal stimulation was found to influence olfactory functioning by altering the perireceptor environment.[60-61] On the other hand, adding an odor to CO_2 facilitates trigeminal perception of CO_2.[54] In general, irritation thresholds are almost always higher than odor thresholds.[62]

3. Physiological effects. Rapid shallow breathing and bronchoconstriction, when objectively measured, are symptoms of *physiological irritation.*[19]

One of the difficulties in the reported research on acetone is that these three types of irritation were not always clearly distinguished and that as a result often the same descriptors were used with different meanings. Moreover, when odor and irritancy threshold levels for acetone were established, acetone concentrations were not always thoroughly checked (see Appendix). Irritation, measured as trigeminal response, very often was not clearly separated from olfactory responses. Similarly, as indicated previously, adaptation effects were not separated from habituation effects.

Studies that were considered adequate with respect to the measurement of acetone concentrations, the methodology used to evaluate health symptoms, and the way of data presentation were used for evaluation and are briefly described in this paper, viz. in 'Subjective measurements of health symp-

toms: use of questionnaires' and/or 'Objective measurements of odor and irritation'. Studies that were reviewed but rejected are indicated in Table 1.[4–9,20,62–67]

III. SUBJECTIVE MEASUREMENTS OF HEALTH SYMPTOMS: USE OF QUESTIONNAIRES

A. Introduction

Neurobehavioral changes include changes in cognitive functioning, affect (mood), and behavior. These may be measured both objectively, or in terms of subjective experience. Neurobehavioral methods therefore include both neurobehavioral (cognitive) tests and questionnaires designed to assess somatic symptoms, mood, and mental health. Neurobehavioral techniques are used in epidemiological studies of long-term effects which compare groups of exposed workers with nonexposed controls, and laboratory (exposure chamber) studies designed to identify the levels at which acute effects occur. The appropriate use of neurobehavioral methods in human studies requires attention to a number of factors related to the objective of the study including the selection of the study design, details of methodology and selected endpoints, analysis of data, and interpretation of results. Many milder health effects or symptoms, such as fatigue, dizziness, nausea, pain, mucosal irritation, and paresthesis, can only be studied through the use of (self-administered) questionnaires or structured interviews.[68] Such methods are increasingly being employed in human studies to investigate the effects of chemicals on the central and peripheral nervous system. These methods are also frequently used for gathering data on past exposures and lifestyle variables. Questionnaires are usually highly structured and specifically designed for a certain study. The quality of the information obtained depends strongly on the validity, that is, reliability, sensitivity, and reproducibility, of the questionnaire, and may be influenced by the individual administering the questionnaire. This section focuses on the assessment of the sensory irritating effects of acetone by means of subjective methods, in particular the use of questionnaires for reporting of exposure-related symptoms.

B. Experimental Studies

1. Controlled Studies on Chemically Naive Subjects

In a laboratory study by Dick et al.,[3] 137 volunteers (18 to 32 years of age) were recruited and tested for (neuro)behavioral performance before, during, and after a 4-h exposure to acetone (250 ppm; purity not indicated), and chemical placebo (5-min 25-ppm exposures to an acetone/methyl ethyl ketone (MEK) mixture presented twice during the 4-h exposure period). The experiment was a mixed model design, with subjects treated as random factor. The Profile of Mood States (POMS) questionnaire was administered as well as a self-made questionnaire, which are designed to obtain information regarding the subjects' exposure perception. The POMS is a factor analysis derived inventory that measures six mood or affective states: tension-anxiety, depression-dejection, anger-hostility, vigour-activity, fatigue-inertia, and confusion-bewilderment. The subjects were instructed to: "include their feelings during the past week including today". This validated and widely used questionnaire consists of 65 five choice adjective items. Results from the self-made questionnaire indicated that the placebo exposure was not associated with increased symptom reporting. The exposure to 250 ppm acetone produced a small but statistically significant change from the control exposure in performance on the anger-hostility scale (men only) of the POMS questionnaire. No data on irritation were reported. It should be noted that no effects on the POMS were reported after

Table 1
Studies Reviewed but Not Used in the Present Evaluation

References	Type of study	Reason for objection
63	Volunteer and dog exposure study (Kinetics)	No measurement of irritancy
64	Worker study (Neurotoxicity)	Very few details on concentration measurement. No measurement of irritancy
65	Worker study (Biological monitoring)	Sampling method not considered to be reliable at higher concentrations (> 250 ppm); No measurement of irritancy
4-5	Volunteer exposure study (Subjective symptoms, measurements in blood and urine)	No details on exposure conditions; Poor description of symptom questionnaire; No tables, figures or other summaries of data
6	Worker exposure study (Subjective symptoms; neurotoxicity)	No details on test measures or time points after exposure; Only frequencies of responding subjects are determined without rating the intensity; No statistical analysis of measures of neurotoxic, irritation, rheumatic or digestive syndromes; Validity of questionnaire and clinical examination questionable
7	Volunteer exposure study (Subjective symptoms)	Calculated concentration; No indication of proper mixing of acetone throughout the large exposure chamber (ca. 44 m3)
20	Worker study (Subjective symptoms)	No information on adsorption/desorption of acetone in the sampling system; No details on number of measurements, number of persons or days sampled, or variation of exposure in time; No direct relation (presented) between measured data and odor perception or irritancy
8	Worker study (Subjective symptoms; biological monitoring)	Insufficient number and very few details on concentration measurements
66	Volunteer and worker exposure study (Kinetics and biological monitoring)	No measurement of irritancy
9	Worker exposure study (Objective and subjective symptoms)	Small experimental groups; No control subjects; No non-solvent related health symptoms included in examination of subjective complaints; Personal samples were reported not to be taken, but data were presented; No correlation found between subjective and objective measures of irritation; psychomotor tests used considered to be invalid by the authors themselves
67	Volunteer exposure study (Biological monitoring)	Few details on exposure conditions; Study not aimed at measurement of irritancy

ingestion of ethanol (95% at 0.84 ml/kg), which was used as a positive control in this study. The absence of any effects in the positive control condition poses questions on the significance of the finding on the anger hostility scale. The same individuals were also exposed to a mixture of acetone at 125 ppm with MEK at 100 ppm, and to MEK alone (200 ppm). No statistically significant effects were detected with the exposures to MEK (200 ppm), and there were no statistically significant interaction effects with the combined acetone/MEK exposures.

Several publications from the group of Seeber[12–18] are based on two laboratory exposure studies with volunteers. These studies were aimed at the evaluation of neurobehavioral effects of acetone. Thirty-two male volunteers (age 19 to 32 years; n = 16 for each experiment) were exposed to 1000 ppm acetone (PA quality), and to noncontaminated room air (control, 0 ppm). (A concentration of 400 ppm ethyl acetate and a mixture of 500 ppm acetone/200 ppm ethyl acetate were used as further exposure conditions but these exposures are not the focus of the present evaluation.) During the first experiment, the exposure lasted 4 h. Four periods of performance testing (simple and choice reaction, memory scanning) and ratings of subjective symptoms (17 item questionnaires) were carried out before, during, and after the exposure. During the second experiment, the exposure lasted 8 h. A break without exposure was introduced after 4 h. The second period of 4 h of exposure was combined with two times 10 min of 50 W bicycle ergometer work load. Five periods of performance and ratings of acute subjective symptoms were carried out before, during (3×) and after the exposure. Ratings of well-being were given every 2 h. "*Well-being*" was measured with a 'paper-and pencil' 7-point analog scale. Four dimensions were evaluated: (1) tension (relaxed - strained); (2) tiredness (awake - tired); (3) discomfort (without complaints - severe complaints); and (4) annoyance (not annoying - very annoying). "*Acute symptoms*" were recorded with a 6-step analog scale ranging from 0 (not at all) to 5 (very often). This computerized 17-item questionnaire is included in the Swedish Performance Evaluation System (SPES) and differentiates four dimensions: (1) discomfort; (2) irritation; (3) tiredness; and (4) difficulties in breathing. It is not clear whether a validation procedure has been carried out for the German language version of these rating scales. Results of the acute symptom questionnaire indicated a significant exposure × time interaction on "irritation" (e.g., of the eyes, throat, or nose). The ratings of irritation, as a sum of those to the eyes, nose and throat, were increased in both laboratory studies (4 h and 8 h) compared with prestudy ratings. The rating of the irritation in the 8-h laboratory study decreased with about 30% during the second half of the exposure. Even 1 h after exposure, its level did not reach the value before exposure or of the control group. There were no indications of any breathing problem. The authors concluded that irritation was present. This was further investigated in detail in a subsequent reanalysis of the data (see further).[69] With regard to the well-being questionnaire, a significant exposure × time interaction was observed for signs of complaints and annoyance. A comprehensive review of these studies, however, is somewhat difficult due to the confusing data presentation and the limited information on the subject characteristics.

In an experimental study, two volunteers were exposed to 6000 or 8000 ppm acetone (purity not indicated), respectively. The ten breaths of 1 l, lasting approximately 30 s, were inhaled by the subjects wearing a nose clip, only with great difficulty, although there was no feeling of irritation. The effects were nausea, suffocation, slight dizziness, and a strong desire to withdraw. Acute exposures of the eyes to acetone vapor were not reported

to produce profound irritation and at a concentration of 10,000 ppm, any irritational effects seemed to be obviated by lachrymation. The author concluded that the subjective assessment of irritancy and unpleasantness was very high.[19]

2. Controlled Studies on Acetone-Exposed Workers

Two laboratory studies of Dalton et al.[1,2] described the assessment of effects of exposure to acetone and evaluated parts of the same dataset. In the first study,[1] 27 acetone-exposed workers in a cellulose fiber production plant (23 females, 4 males; mean age 38 years, range 23 to 65; of which 14 smokers) and 27 matched controls (not further indicated) were individually exposed for 20 min to 800 ppm acetone (purity >99.5%). Response bias was accounted for by exposure of the subjects to 200 ppm phenylethyl alcohol (PEA), a nonirritant control odorant. The workers had been at their jobs for at least 1 h (and no more than 5 h). It was noted that workers and controls were tested at different locations, indicating that the groups were not homogeneous. Immediately after each exposure session, subjects were asked to complete a questionnaire in which they rated the occurrence and/or intensity of a variety of health symptoms. The health symptom questionnaire (adapted from Hudnell et al.[70]) used the validated Labelled Magnitude Scale to obtain ratings for 7 solvent-associated symptoms, 14 somatic (control) symptoms, and a rating of irritation at the moment of answering the questionnaire. Occupational exposure to acetone significantly reduced the perceived odor intensity of 800 ppm acetone relative to the perception of the nonexposed controls. The workers rated the odor of acetone as weak to moderate, whereas controls rated the odor as strong to very strong. Responses to the perceived irritation mirrored the results for odor, viz. workers perceived the irritation (nasal and throat) from acetone significantly different from the controls. Workers rated the perceived irritation as barely detectable to weak, whereas controls rated the perceived irritation as strong. The study showed that the perception of odor intensity and the degree of an individual's bias to report irritation and the exposure history are very important factors mediating the reported levels of irritation and health symptoms.

In the second laboratory study,[2] using the same methodology with respect to questionnaires, the emphasis was on the influence of cognitive bias on perceived odor, irritation, and health symptoms. Naive volunteers (sex not indicated; mean age 33.7 years, range 25 to 64; smoking status not indicated; 30/group) were positively, negatively, or neutrally informed about the consequences of exposure to acetone. They were subsequently exposed to 800 ppm acetone (purity >99.5%) for 20 min or to 200 ppm PEA and tested in the same way as in the first study. Subjects in the positive bias condition significantly perceived the odor of acetone as weaker during the exposure and reported significantly fewer health symptoms (barely detectable to weak nasal and throat irritation, lightheadedness, nausea and drowsiness) than subjects in the neutral and negative bias condition. The study provides evidence that the perceived odor and irritation after exposure to acetone can be modulated by information about the consequences of long-term exposure to this chemical. Variables most highly correlated with perceived irritation were the perceived odor intensity and the perceived irritation from the no-irritant control odorant PEA. These studies indicate that perception of odor intensity and response bias may significantly influence reported perception of irritation due to acetone exposure.

An experimental study by Wysocki et al.[24] was carried out to establish both olfactory and sensory irritation thresholds of ac-