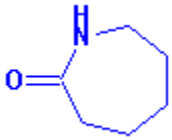
CAPROLACTAM
(AEROSOL, VAPOR & PARTICULATE)

(Aminocaproic lactam; epsilon-Caprolactam; Hexahydro-2H-azepin-2-one; 2-Oxohexamethylenimine; 2-Ketohexamethylenimine)
CAS 105-60-2



1. Summary

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360 (b) (2)). OEHHA developed a Technical Support Document (TSD) in response to this statutory requirement that describes acute, 8-hour and chronic RELs and was adopted in December 2008. The TSD presents methodology reflecting the latest scientific knowledge and techniques, and in particular explicitly includes consideration of possible differential effects on the health of infants, children and other sensitive subpopulations, in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, chapter 731, statutes of 1999, Health and Safety Code Sections 39669.5 *et seq.*). These guidelines have been used to develop the following RELs for caprolactam: this document will be added to Appendix D of the TSD.

Exposure to caprolactam has been found to cause upper respiratory and eye irritation in both animals and humans. Exposure causes inflammation of the nasal and laryngeal epithelium in exposed rodents. The site-of-contact nature of the lesion to the most sensitive cells lining the upper respiratory tract indicates that caprolactam is primarily a direct-acting irritant, rather than a chemical requiring metabolic activation in nasal mucosa to cause tissue injury. Although there is no evidence for reproductive or developmental effects at levels that produce sensory irritation, considerably higher doses administered orally to pregnant rats have resulted in reduced weight of offspring. Literature summarized and referenced in this document covers the relevant published literature for caprolactam through Fall 2011.

1.1 Acute REL Summary

Acute 1-Hour inhalation reference exposure level	**50 µg/m^3 (11 ppb)**
Critical effect(s)	Increased eye blink frequency in humans

Hazard index target(s)	Eyes

1.2 8-Hour REL Summary

8-Hour inhalation reference exposure level	**7 µg/m^3 (1.4 ppb)**
Critical effect(s)	Inflammatory changes of nasal and laryngeal epithelium in rodents
Hazard index target(s)	Upper respiratory system

1.3 Chronic REL Summary

Chronic inhalation reference exposure level	**2.2 µg/m^3 (0.5 ppb)**
Critical effect(s)	Inflammatory changes of nasal and laryngeal epithelium in rodents
Hazard index target(s)	Upper respiratory system

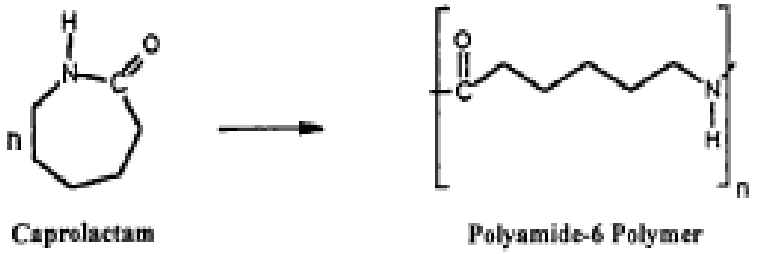
2. Physical and Chemical Properties [from HSDB (2006), unless noted otherwise]

Description	A semi-volatile white, hygroscopic, crystalline solid or flakes with unpleasant odor
Molecular formula	$C_6H_{11}NO$
Molecular weight	133.16 g/mol
Density	1.05 g/cm^3 @ 25 °C
Boiling point	270 °C
Melting point	69.3 °C
Vapor pressure	0.0011 mm Hg @ 20 ºC (68ºF) 0.0021 mm Hg @ 25 °C (77ºF), saturated vapor concentration = 13 mg/m^3 0.153 Pa @ 20 ºC, and 0.275 Pa @ 25 °C (Zaitsau et al., 2006)
Odor threshold	≤0.15 mg/m^3 (Ziegler et al., 2008)
Solubility	Very soluble in water, benzene, diethyl ether, and ethanol. Soluble in chlorinated solvents, petroleum distillates, and cyclohexene
Conversion factor	1 ppm = 4.63 mg/m^3 (as vapor) @ 25 °C

3. Occurrence and Major Uses

Caprolactam is the monomer used in the polymerization process to manufacture synthetic fibers and resins, known as Nylon-6 (Cooper et al., 1993; NPG, 2007). Nylon-6 was first developed for commercial use in the 1930's. This type of nylon has also been called polyamide-6, which refers to the type of polymer produced by the interaction of an amino group of one caprolactam molecule and a carboxylic group of another caprolactam molecule to give a protein-like structure. The reaction, shown in Figure 1, entails a ring-opening polymerization:

Figure 1. Ring opening polymerization of caprolactam (Lander, 2002)



Nylon-6 is the most widely used type of nylon, representing more than 60% in volume of all nylon types used worldwide (NPG, 2007). U.S. EPA (2006) notes in their IUR (Inventory Update Reporting) that the aggregated national production volume of caprolactam was 1 billion lbs and greater in 2006. Nylon-6 is found in many products, such as in:

- Carpets, rugs and home textiles
- Engineering plastics (automotive, E&E, equipment housing)
- Tire cord (for reinforcement of heavy-duty tires)
- Textiles (apparel, hosiery, lingerie, sportswear, swimwear, casual wear, fashion wear, socks, umbrellas, luggage, tents, parachutes, sleeping bags, etc.)
- Film (food packaging, industrial packaging, medical applications)

Bongard (2000) reported that about 75% of manufactured Nylon-6 is used in fibers (textile, industrial, carpet) and 25% in engineering plastics and food packaging film. It was expected that this ratio would gradually change more in favor of plastics and film over time. Minor uses of caprolactam include use as a solvent in paint, and for the synthesis of the amino acid lysine (IARC, 1999; Bradley et al., 2004). Another common type of nylon known as Nylon-6,6 is produced from hexamethylene diamine and adipic acid and does not contain caprolactam (Hegde et al., 2004).

In industrial processes, exposure occurs during the production of the caprolactam monomer when the monomer is polymerized, extruded, chilled, or cut into pellets, or when the polymer pellets are re-melted, re-extruded, and drawn into fiber or film (ACGIH, 2003). Exposure may also occur during recycling of waste Nylon-6

carpets, which can be reprocessed in full back to its raw material (NPG, 2007). The monomer vaporizes from the heated processes and condenses as fume (i.e., a cloud of small particles suspended in air) (ACGIH, 2003). In early industrial studies, in which high concentrations of caprolactam were recorded in workplace air, contact of the fume with cooler surfaces resulted in formation of light feathery flakes (Hohensee, 1951; Kelman, 1986). Exposure is primarily as an aerosol, although the vapor form would also be expected to be present at concentrations relevant to the RELs (ACGIH, 2003). In the ambient air, caprolactam has been observed as a fine aerosol collected on $PM_{2.5}$ filters as a result of the probable release from a facility that used caprolactam as a raw material (Cheng et al., 2006). Wilkins et al. (1993) found caprolactam in floor dust following a thermal desorption process to analyze VOC emission profiles. Thus, caprolactam also appears to adsorb onto dust particles.

Measurable levels of caprolactam have been found primarily in indoor air as a result of release of the vapor or particulate from carpeting containing Nylon-6 (IWMB, 2003). Caprolactam may also migrate into foods packaged in Nylon-6 film (Bradley et al., 2004). Caprolactam was detected in foodstuffs packaged in Nylon-6 in the range of 2.8 to 13 mg/kg.

The polymerization process of caprolactam to nylon polymer is not 100 percent efficient, thus allowing some of the un-polymerized caprolactam into the final product. Goldblatt et al. (1954) noted that the polymerized fiber contains approximately one percent of the unreacted caprolactam monomer. A more recent study suggests that total caprolactam contaminants, plus lesser amounts of its various oligomers, are present at 1% or less in some Nylon-6 products (Venema et al., 1993). These oligomers found following polymerization include cyclic oligomers (i.e., the cyclic dimer, trimer and tetramer, etc.), as well as some linear oligomers (Krajnik et al., 1982; Ballistreri et al., 1987; Bonifaci et al., 1991; Venema et al., 1993). No investigations regarding the health effects of caprolactam oligomers could be located in the literature. *In vitro* cytotoxicity testing with polymers and their corresponding monomers, one of which was the monomer vinylcaprolactam, showed that the monomers can have much greater cytotoxicity with respect to the corresponding polymers (Vihola et al., 2005). The authors stated that the polymers tested in this study were 156,000 to 1,500,000 g/mol. Whether caprolactam and its oligomers would show a similar pattern of cytotoxicity was unclear from this study.

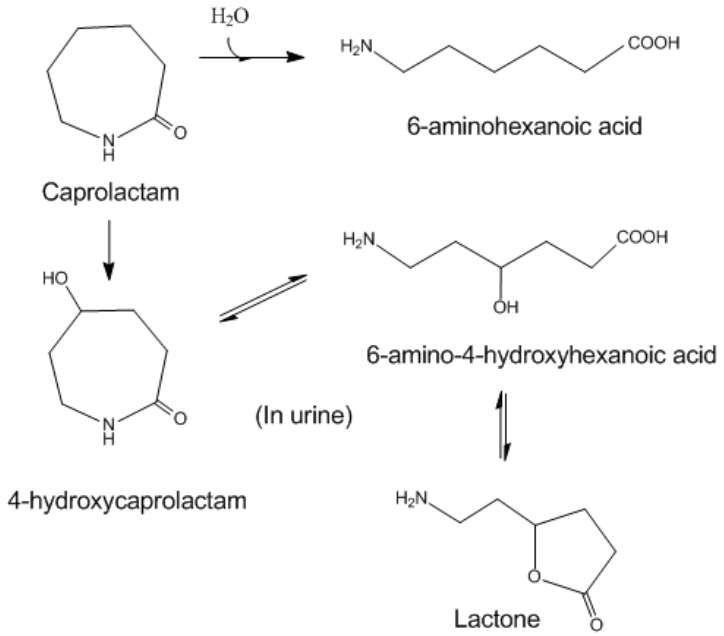
Based on the measured emission rate of caprolactam from carpet samples, modeled air concentrations for office and classroom scenarios ranged from 39 to 450 $\mu g/m^3$ (IWMB, 2003). A chamber study found caprolactam emissions from some polyamide carpets resulted in chamber concentrations ranging from 6 to 97 $\mu g/m^3$ on the 28th day of chamber testing (Wilke et al., 2004). In indoor monitoring studies, caprolactam was detected in all floor dust samples collected during an indoor air study in nine public buildings (Wilkins et al., 1993). In another study, the average caprolactam concentration in a new California portable classroom

during school hours over 8 weeks following installation of a Nylon-6 broadloom carpet was 22.2 µg/m^3 (range: 10.6 - 30.1 µg/m^3) (Hodgson et al., 2004). The emission rate of caprolactam following installation of the carpet was about 5 mg/h prior to occupancy, and dropped to 3 mg/h 27 weeks after first occupancy. Similar portable classrooms that installed upgraded carpets containing Nylon-6,6 emitted low to non-detectable concentrations of caprolactam (maximum: 1.4 µg/m^3).

4. Metabolism

In rats, Kirk et al. (1987) observed that approximately 16% of ingested caprolactam in diet was excreted in urine as 4-hydroxycaprolactam and a small amount as the non-hydroxylated acid, 6-aminohexanoic acid (Figure 2). Following a single oral dose of [^{14}C]caprolactam in male rats, 77.6% of the radioactivity was excreted in urine, 3.5% in the feces, and 1.5% in the expired air in 24 hrs (Unger et al., 1981). The half-life of disappearance of radioactivity from the blood was 3.0 hr. Similar to the findings by Kirk et al., Unger et al. (1981) observed two metabolites following oral administration, which comprised 79.3 and 17.7% of the total urinary radioactivity. However, Unger et al. made no attempt to identify these urinary metabolites. Unchanged caprolactam represented only 2.3% of the total urinary radioactivity. The radiolabeled caprolactam was widely distributed among the tissues of the rat, including the brain, with concentrations mostly similar to that in the blood. The radioactivity was consistently lower in fat relative to the blood in the first 24 hrs, indicating a low affinity of caprolactam and its metabolites for adipose tissue.

Figure 2. Metabolism of caprolactam



Oral delivery of [^{14}C]caprolactam in male and female mice also showed that the chemical is rapidly absorbed from the stomach and freely distributed into all tissues (Waddell et al., 1984). Almost all radioactivity was eliminated in 24 hours, although some retention of radioactivity during this time was noted in the brain, nasal epithelium, olfactory lobe of the brain, liver, optic lens and Harderian gland. In pregnant mice, sites of localization of the radiolabel were similar to non-pregnant mice, with the exception that some residual radioactivity was also noted in the umbilical cord, amnion, and yolk sac. No radioactivity was retained in any other fetal tissues. It was speculated that metabolism of caprolactam in the nasal tissue may produce a metabolite that was slow to clear. The apparent retention of radioactivity in the olfactory bulb was thought to represent an artifact caused by the washing of radioactivity from the nasal epithelium by hexane during the freezing procedure.

5. Acute Toxicity of Caprolactam

5.1 Acute Toxicity to Adult Humans

Occupational exposure to caprolactam emitted during Nylon-6 processes is known to cause acute eye and upper respiratory tract irritation (Hohensee, 1951; Ferguson and Wheeler, 1973; Kelman, 1986). Dermal irritation and dermatitis also occur in occupational settings with short-term and repeated exposure to the solid form of caprolactam, or caprolactam particulate that has “condensed” (sic) onto surfaces from the airborne vapor or aerosol phase (Hohensee, 1951; Ferguson and Wheeler, 1973; Kelman, 1986; NIOSH, 1995a).

Two published studies, an industrial observational exposure study by Ferguson and Wheeler (1973), and a chamber controlled human exposure study by Ziegler et al. (2008) examined the acute sensory irritant effects resulting from directly measured concentrations of caprolactam. These two studies are summarized and assessed for REL development below. No peer-reviewed studies have been conducted specifically examining toxicological effects of caprolactam released from finished products that contain the chemical (e.g., finished Nylon-6 carpets).

Additionally, several case reports of workers heavily exposed to caprolactam developing seizures are summarized in this section. This is a particular concern given that, in general, children are more vulnerable than adults to the neurologic effects of chemicals. Because caprolactam is a respiratory irritant, the available data for the potential of caprolactam also acting as a sensitizing agent for allergic responses is presented. Lastly, the basis for caprolactam occupational exposure limits (e.g., ACGIH and NIOSH) is summarized at the end of this section.

Ferguson and Wheeler (1973)

Ferguson and Wheeler (1973) exposed 5 healthy unacclimated male worker “volunteers” at a caprolactam polymerization plant. Exposures were to

caprolactam vapor at concentrations of 10, 14, 25, and 104 ppm (46, 65, 116, and 482 mg/m^3, respectively) while subjects were standing or conversing for several minutes downwind from a known emission source. The smoking status of the workers was not reported. 'Unacclimated' was defined as workers who were experienced employees of the work environment, but reportedly were not continuously exposed in their ordinary duties. Although the authors report exposure was to the "vapor" form of caprolactam, the concentrations were above the saturated vapor concentration of 13 mg/m^3, indicating that exposure included caprolactam aerosol.

Air sampling for the exposures was area level rather than personal monitoring and was set at a height of about 60 inches from the floor and was averaged over a minimum of 30 minutes (Ferguson and Wheeler, 1973). The instrumentation for these exposures was presumably the same used for estimating 8-hour time weighted average concentrations in the second part of the study: a liquid absorption train that consisted of three flasks in series set up to collect the caprolactam in measured volumes of air. The flasks were half-filled with water and connected with one another by fritted glass delivery tubes. The train was connected to a fixed vacuum supply through a wet test meter. A similar arrangement was also employed that used gas traps rather than flasks, and a portable vacuum pump was used as a vacuum source. Two-mm glass beads were used in the first trap as a sparger. Both sampling techniques were considered effective, as no caprolactam was found in the last trap in either train. Analysis was by gas chromatography (GC) with a flame ionization detector.

The sampling and analytics methods above are presented in detail because of the implications for error in exposure assessment. This air sampling technique was a commonly used method in the 1970's, but some particulates could have been missed or lost due to the use of fritted glass (Gill, 2011). Further, using GC for analysis of caprolactam is less sensitive than using high pressure liquid chromatography (HPLC) because the solvent peak tends to interfere with detection of caprolactam (Nau et al., 1984; Gill, 2011). Current methods for caprolactam air sampling recommended by OSHA include XAD resins or other absorbents, and analysis using HPLC and mass spectrometry (OSHA, 1988).

Most or all of the subjects in the Ferguson and Wheeler study reported transient nasal and throat irritation at all concentrations, including 4 out of 5 individuals exposed to 10 ppm (46 mg/m^3). Eye irritation was noted only in one volunteer at the highest concentration. The authors did not indicate whether throat irritation was a result of mouth-breathing (due to the unpleasant odor and/or nasal irritation) by those exposed. The degree of discomfort felt by the workers was considered dose-responsive, but was not quantified, reportedly due to wide differences in the degree of discomfort among the individual subjects. Thus, scalar functions that had been used in an attempt to evaluate degrees of discomfort were not presented. Some of the study participants also were exposed to similar concentrations for up to 30 min, but the sensory effects were not clearly

stated or quantified. Brief exposure to 400-1200 ppm caprolactam was described as “extremely irritating”, resulting in a “choking” response.

A similar acute exposure study was arranged at a caprolactam monomer plant, although specific conditions of the exposure were not presented by the authors (Ferguson and Wheeler, 1973). In that part of the study, 14 ppm (65 mg/m^3) did not result in distress or discomfort. The authors speculated that the conditions of 100% humidity at the monomer plant (the polymer plant was described as having near normal humidity) may have been a factor in reducing sensory irritation. The authors also noted that caprolactam concentrations at the monomer plant appeared to be more uniform, suggesting that the greater variability in the concentration at the polymer plant might have resulted in brief high exposures leading to sensory irritation. The authors, however, did not present quantitative data documenting the variation in caprolactam concentration during the acute exposures.

Ferguson and Wheeler concluded that the irritant response threshold for the workers was at or near 10 ppm (46 mg/m^3) caprolactam, and that 5 ppm (23 mg/m^3) would be 50% of the discomfort threshold and somewhat below the no-effect level. The authors further state that additional support for their worker threshold value of 5 ppm is based on their findings of no reported distress among the employees in active and semi-active areas at concentrations up to about 7 ppm, although this did not appear to reflect responses to a systematic survey of the workforce (discussed in Section 6.1).

Ziegler et al. (2008)

Ziegler et al. (2008) conducted chamber exposures of 20 adult subjects (10 men and 10 women) to 0, 0.15, 0.5, and 5 mg/m^3 caprolactam vapor for 6 hours on 4 successive days. The stated study goal was to address possible chemosensory effects of caprolactam at low concentrations reflecting the indoor environment. Chemosensory subjective effects were assessed by a standardized questionnaire. Objective measures of exposure were assessed by eye blink frequency using a standard manual counting procedure and a new semi-automated method; conjunctival hyperemia (eye redness) based on digital slit lamp photographs taken during exposure; and by nasal resistance using active anterior rhinometry before and after exposures. Except for nasal resistance, the questionnaire and objective tests were given at time 0 (i.e., just after entering the chamber), 1 hr, 3 hrs and 6 hrs during exposure. Nasal resistance was measured only at baseline and once more at the end of the exposure period at 6 hrs.

Two techniques were used to determine eye blink frequency at each time point during caprolactam exposures, a manual count method followed immediately by a semi-automated approach. For the manual method, Ziegler et al. digitally recorded the eyes of the participants for a specified duration under dim lighting conditions. The recording was later reviewed in a double blind fashion and the

number of eye blinks manually recorded. The second method involves a neon light shining on the eye surface of the participants and a sensor records the change in beam length that reflects back when the participant blinks. The authors noted in their report that the semi-automated method is still in the testing phase and its applicability and reproducibility needs to be verified in further studies. The manual method is a traditional procedure used in many studies to record eye blinks. OEHHA concurred with Ziegler et al. that for eye blink frequency, greater confidence should be placed on the findings using the standard manual method.

The questionnaire consisted of 29 items related to sensory irritant symptoms and perceptions (e.g., odor). This multi-item battery was used to generate a total daily score. Six adjectives were presented for rating the intensity of symptoms on a Likert-type scale from zero (not at all) to 5 (very severe). The 29 items were also categorized into seven subscores for assessment. The subscores include: 1) non-specific symptoms: *feeling of weakness, headache, dizziness, felling of being unwell*; 2) not classified: *blurred vision, irritation to the throat, skin irritation*; 3) sensations of bad taste: *very unpleasant taste in the mouth, unpleasant taste, foul taste*; 4) respiratory symptoms: *pressure on the chest, coughing, dyspnea*; 5) olfactory symptoms: *perception of bad air, foul smell, unpleasant smell, stink*; 6) nasal irritation: *nasal irritation, itching nose, dry nose, runny nose, burning nose*; 7) irritation to the eyes: *tiredness of the eyes, itchy eyes, burning eyes, eye irritation, dry eyes, watery eyes, redness of the eyes*. A second section evaluating well-being (i.e., tension, tiredness, annoyance and general well-being) was rated on a visual analog scale from one (no symptoms) to seven (severe symptoms).

Ziegler et al. (1998) found that caprolactam exposure was associated with a statistically significant increase in the subjects' detection of an unpleasant odor beginning at 0.15 mg/m^3. However, at 5 mg/m^3 the average intensity score of 1.2 was only slightly pronounced (i.e., between "barely" (1) and "somewhat" (2) for an odor nuisance). Subscores for eye and nasal irritation and eye/nasal irritation combined showed no statistically significant concentration-response relationships using the analysis of variance (ANOVA) test. Discounting odor nuisance, there were no statistically significant differences among the other individual symptoms and subscores associated with caprolactam exposure. At 5 mg/m^3 the total symptom score based on all 29 acute symptom items was significantly elevated ($p \leq 0.05$). No statistically significant increase or decrease in the total symptom score was observed in the course of the day. Thus, these results do not indicate any adaptation or habituation processes in the course of the 6 hour exposure.

For the three objective measures of sensory irritation, the authors found no statistically significant concentration-response relationships by the ANOVA test, but noted there was a "non-significant trend" towards higher blink frequency and nasal resistance associated with increasing caprolactam concentrations. As noted below, there appear to be statistical issues with these analyses that could have obscured effects of caprolactam.

Because there was little variation over time among most of the subjective and objective measures during the six hours of exposure, the authors had combined the data collected at time 0 (i.e., just after entering the chamber), 1 hr, 3 hrs and 6 hrs for their statistical analyses. OEHHA obtained the individual raw data, kindly provided by Dr. Ziegler, with the primary goal of running several statistical analyses on the data collected at 1 hour of exposure, the exposure duration that forms the basis of an acute REL, as well as the other time points. The combined data collected up to 6 hrs of exposure were also analyzed by OEHHA.

For statistical evaluation of the raw data, we used the non-parametric Page's trend test to verify any associations between caprolactam exposure among both the objective and subjective endpoints. In the Page's trend test, the response for each ordered category (caprolactam concentration in this case) is ranked for each participant. Ranking is based on an ordinal scale (i.e., 1, 2, 3 and 4 since there are four exposure concentrations). For example, over ordered doses, if a person's blink response is 24, 15, 70 and 90 blinks/90 sec for the 0, 0.15, 0.5 and 5 mg/m^3 caprolactam concentrations, respectively, then the participants' ranks are 2-1-3-4. The ranks for each dose are then summed over all the individuals and those sums are used to calculate an L-statistic and corresponding p-value to determine if an association between caprolactam concentration and eye blink frequency exists.

Ziegler et al. originally used a parametric repeated measures ANOVA for their statistical analysis of normally distributed data and a non-parametric Kruskal-Wallis ANOVA for non-normally distributed data, although they did not specify which outcomes each was applied to in every situation. To avoid any assumptions of normality in this data, we chose non-parametric methods. For this set of data, Page's trend test is a more appropriate non-parametric method for assessing the possibility of a dose-response relationship. This is because Page's test is able to use the order of the dose categories to assess whether a general trend in response is present over increasing dose, which is essentially the question of interest. Moreover, Page's trend test accounts for multiple measurements of the same subjects at different exposure times (i.e., repeated measures) and the within-subject correlation in values that such a design creates, whereas the Kruskal-Wallis test does not. In the instances where a statistically significant trend ($p \leq 0.05$) was found, we utilized the Wilcoxon signed-rank test to identify any statistically significant differences at $p \leq 0.05$ between the control exposure and each dose group.

Table 1 presents the statistical results for eye redness and nasal resistance based on Page's trend test. No statistically significant dose-response relationship was found for the eye redness score at 1 hour of exposure. In addition, no dose-response relationship was found at the other time points of 0, 3 and 6 hours (data not shown). Nasal resistance was also not statistically significantly affected by the 6 hour exposure to caprolactam. Nasal resistance values in Table 1 represent the difference between resistance just before entering the exposure chamber and

resistance at the end of the 6 hr exposures. A negative mean for nasal resistance (0 and 0.15 mg/m^3 caprolactam exposures) indicates resistance was less at the end of 6 hr exposure than before entering the chamber. In addition to mean, standard deviation, and interquartile range (IQR), mean rankings for each dose group are shown because the rankings are what Page's test ultimately utilizes. Though the L-statistic is calculated using the sum of ranks for each dose group, the mean is provided here to keep ranks on the original ordinal 1 to 4 scale for ease of comprehension.

Table 1. Page's trend test results (mean ± standard deviation, median, interquartile range and mean rank) for eye redness at 1 hour of caprolactam exposure, and for nasal resistance at 6 hours of caprolactam exposure, performed by OEHHA

Outcome & Statistic	Caprolactam concentration (mg/m^3)				Page's trend test result
	0	0.15	0.5	5.0	
Eye redness score[a]					
Mean±SD	2.00±0.00[b]	2.00±0.00[b]	2.00±0.00[b]	2.05±0.15	*p*=0.64
Median	2	2	2	2	
IQR	0	0	0	0	
Mean rank	2.45	2.45	2.45	2.65	
Nasal resistance[c]					
Mean±SD	-0.049±0.21	-0.029±0.22	0.013±0.12	0.020±0.12	*p*>0.99
Median	0.00	0.04	0.03	0.01	
IQR	0.14	0.09	0.09	0.09	
Mean rank	2.45	2.45	2.75	2.35	

[a] Score ratings for eye redness were: (1) very slight, (2) slight, (3) moderate, (4) severe
[b] No standard deviation or IQR because all subjects rated a (2) for slight eye redness
[c] Nasal resistance presented in units of kPa/L/sec. Nasal resistance values in Table 1 are different from those presented in the original paper by Ziegler et al. (2008). The correct formula for calculating nasal resistance used in Table 1: LRxRR/(LR+RR) was inadvertently calculated as LRxRR/LR+RR in the original paper, where LR is left nostril resistance and RR is right nostril resistance (A. Ziegler, personal communication). Nevertheless, use of either formula resulted in the same outcome: no effect of caprolactam on nasal resistance.

Based on the manual method for recording eye blinks, the Page's trend test confirmed a statistically significant association for increasing caprolactam exposure and eye blink frequency at 1 hour of exposure (Table 2). The Wilcoxon sign-rank test showed that exposure to 5 mg/m^3 caprolactam resulted in a statistically significant increase (p=0.01) in eye blink frequency compared to the control group. A concentration-response association was not observed at 0 hour (defined by Ziegler et al. as within 5 minutes after entering the chamber). For reasons not specified in the Ziegler study, only 4 to 8 individuals were assessed

by the manual eye blink count method at each caprolactam concentration during the 3 and 6 hour exposure effects assessment. OEHHA did not consider this to be a sufficient number of individuals for statistical analysis for these exposure durations.

Applying Page's trend test to the novel semi-automated approach for estimating eye blink frequency, a concentration-response association was not observed at 0 or 1 hour of exposure (Table 2). A statistically significant association for increasing caprolactam exposure and eye blink frequency was observed at 3 and 6 hours of exposure, and for the 0, 1, 3, and 6 hour eye blink data when they were combined. In all cases when an association was found at $p<0.05$ using the Page's trend test, the Wilcoxon sign rank test observed a statistically significant difference ($p<0.05$) between the control exposure and the 5 mg/m^3 exposure. No other statistically significant comparisons between exposure concentrations were found.

Table 2. Page's trend test results (mean ± standard deviation (SD), median, interquartile range (IQR) and mean rank) by OEHHA for eye blink frequency

Exposure time & Statistic	Caprolactam concentration (mg/m^3)				Page's trend test result
	0	0.15	**0.5**	**5.0**	
Manual method					
0 hr[a]					
Mean±SD	25.6±19.9	27.3±23.2	24.0±18.1	30.4±23.1	*p*=0.88
Median	18.5	18.0	19.0	26.0	
IQR	15.5	22.5	15.8	28.0	
Mean rank	2.53	2.48	2.38	2.63	
1 hr					
Mean±SD	18.7±11.8	25.2±24.6	23.5±14.7	34.4±29.2	*p*=0.002
Median	17.0	18.0	21.5	26.0	
IQR	12.8	22.0	19.0	29.8	
Mean rank	1.90	2.38	2.58	3.15[b]	
Semi-automated method					
0 hr[a]					
Mean±SD	21.9±14.4	25.2±24.2	23.8±20.4	28.4±20.6	*p*=0.15
Median	20.5	17.5	19.5	27.0	
IQR	10.5	18.5	13.8	24.5	
Mean rank	2.38	2.28	2.40	2.95	
1 hr					
Mean±SD	18.0±10.5	25.5±25.3	22.8±14.6	29.7±25.8	*p*=0.23
Median	14.0	20.5	19.0	21.5	
IQR	13.5	29.0	14.0	27.3	
Mean rank	2.15	2.63	2.53	2.70	
3 hr					
Mean±SD	21.5±21.0	19.4±14.9	18.2±13.0	29.1±25.0	*p*=0.01
Median	15.5	17.5	16.5	22.5	
IQR	16.5	14.5	11.3	20.5	
Mean rank	2.18	2.28	2.28	3.28[c]	
6 hr					
Mean±SD	16.5±11.4	21.2±19.7	18.9±18.1	25.9±21.8	*p*<0.001
Median	14.5	16.0	13.0	15.5	
IQR	15.5	22.0	14.8	22.3	
Mean rank	1.88	2.30	2.28	3.55[d]	
Combined					
Mean±SD	19.5±10.8	22.8±20.0	20.9±15.2	28.2±21.0	*p*=0.02
Median	17.3	18.4	15.8	23.3	
IQR	9.1	17.3	10.0	22.5	
Mean rank	2.28	2.30	2.10	3.33[e]	

[a] "0 hour" exposure means eye blink data collection started within 5 minutes after entering the chamber
[b] Sign rank test: control (0 mg/m^3) different from 5 mg/m^3 group (*p*=0.01)
[c] Sign rank test: control (0 mg/m^3) different from 5 mg/m^3 group (*p*=0.02)
[d] Sign rank test: control (0 mg/m^3) different from 5 mg/m^3 group (*p*=0.01)
[e] Sign rank test: control (0 mg/m^3) different from 5 mg/m^3 group (*p*=0.01)

For the subjective questionnaire subscores at one hour of exposure, we found a

statistically significant concentration-response relationship (p=0.02) for perceived eye irritation (Table 3) by the Page's trend test, consistent with the objective eye blink measure. The 5.0 mg/m^3 exposure differed from the control group (p<0.05) by the sign-rank test. A statistically significant trend was also verified at 6 hours of exposure, but the ensuing sign-rank test comparing control vs. 5.0 mg/m^3 groups was not statistically significant (data not shown). There was no concentration-response relationship at 0 or 3 hours (data not shown).

Combining the 0, 1, 3 and 6 hr eye irritation subscore measurements for statistical analysis, as Ziegler et al. (2008) had done in the original report, also yielded a statistically significant association by the Page's trend test (p=0.002) (data not shown in Table 3). Using the sign-rank test, the control group was different from both the 5 mg/m^3 group (p=0.01) and the 0.5 mg/m^3 group (p=0.03).

The subjective nasal irritation score at one hour of exposure did not demonstrate a statistically significant concentration-response relationship at 1 hour of exposure (Table 3). No statistically significant concentration-response was observed at any of the other time points (0, 3 and 6 hours), or when all the time point data was combined (data not shown in Table 3).

Table 3. Page's trend test results (mean ± standard deviation (SD), median, interquartile range (IQR), and mean rank) performed by OEHHA for selected subjective questionnaire results at 1 hour of exposure: eye and nose irritation subscores, odor nuisance subscore, and total symptom and complaint score of 29 questions with and without odor subscore[a].

Outcome & Statistic	Caprolactam concentration (mg/m^3)				Page's trend test result
	0	0.15	0.5	5.0	
Eye irritation					
Mean±SD	0.18±0.24	0.26±0.50	0.34±0.48	0.36±0.44	*p*=0.02
Median	0.14	0.00	0.21	0.21	
IQR	0.29	0.18	0.32	0.46	
Mean rank	2.13	2.18	2.83	2.88[b]	
Nose irritation					
Mean±SD	0.14±0.23	0.18±0.28	0.18±0.31	0.24±0.33	*p*=0.42
Median	0	0	0	0	
IQR	0.25	0.20	0.20	0.45	
Mean rank	2.30	2.53	2.53	2.65	
Odor nuisance					
Mean±SD	0.10±0.22	0.20±0.30	0.24 ±0.30	1.09±0.90	*p*<0.001
Median	0	0	0	1	
IQR	0	0.31	0.50	0.94	
Mean rank	1.83	2.15	2.40[c]	3.63[c]	
Total score					
Mean±SD	0.13±0.16	0.19±0.33	0.21±0.26	0.37±0.32	*p*<0.001
Median	0.07	0.07	0.12	0.22	
IQR	0.16	0.13	0.16	0.44	
Mean rank	1.83	2.15	2.68	3.35[d]	
Total score w/o odor					
Mean±SD	0.13±0.17	0.18±0.36	0.20±0.28	0.26±0.31	*p*=0.003
Median	0.08	0.06	0.10	0.12	
IQR	0.19	0.07	0.09	0.28	
Mean rank	2.00	2.18	2.73	3.10[e]	

[a] Score ratings were: (0) not at all, (1) barely, (2) somewhat, (3) quite pronounced, (4) severe, and (5) very severe
[b] Control (0 mg/m^3) group different from 5 mg/m^3 group by the sign-rank test (*p*=0.02)
[c] *Control* (0 mg/m^3) group different from the 5 mg/m^3 (*p*<0.001) and 0.5 mg/m^3 group (*p*=0.04) by the sign-rank test
[d] Control (0 mg/m^3) group different from 5 mg/m^3 group by the sign-rank test (*p*<0.001)
[e] Control (0 mg/m^3) group different from 5 mg/m^3 group by the sign-rank test (*p*=0.01)

For the odor subscore, a significant concentration-response relationship was observed (Table 3). Ziegler et al. observed a difference between no exposure and all three exposure groups when odor response data collected at 0 (just after entering the chamber), 1, 3, and 6 hrs of exposure were combined. Utilizing just the one hour questionnaire data, however, we observed a statistically significant difference only for the 0.5 and 5.0 mg/m^3 exposures compared to the control exposure.

The total symptom and complaint score was evaluated both with and without the odor responses. The latter analysis was performed in order to evaluate how strong a driver odor was for the total subjective complaint score. Ziegler et al. had observed a statistically significant difference at $p<0.05$ between the non-exposed and the 5 mg/m^3 caprolactam exposure groups when the questionnaire data (including the odor questions) was combined from all four time points. Using Page's trend test, OEHHA found a statistically significant concentration-response relationship at 1 hour of exposure, when the odor subscore was included (Table 3). The sign-rank test showed a difference in response between the control and the 5 mg/m^3 exposure ($p<0.001$). When the odor questions were excluded, there was still a significant concentration-response relationship at one hour of exposure ($p=0.003$). The sign-rank test for odor-excluded scores indicated a statistically significant difference between the control and 5 mg/m^3 exposures ($p=0.009$).

A benchmark concentration analysis was carried out by OEHHA using U.S. EPA (2009c) continuous modeling methodology on the eye blink frequency data (Table 2) and eye irritation data from Table 3. For the eye blink and eye irritation datasets, continuous modeling demonstrated a significant dose-related trend ($p<0.05$, test for difference among dose groups), but was unable to provide a fit to the data (chi-square test for goodness of fit, where $p\geq0.10$ for acceptability) using the available models. Specifically, the models could not be fit to the data because one or more of the observed means was not positioned reasonably close enough to the estimated means.

OEHHA applied the Spearman rho test to look for correlations in response to caprolactam concentration between the eye blink frequency and subjective eye irritation score at 1 hour of exposure. First, individual caprolactam responses were characterized by comparing response at the 5 mg/m^3 dose to response at the baseline 0 mg/m^3 dose. For subjective eye irritation, an absolute measure of response was calculated by simply subtracting an individual's control dose response from their high dose response. For eye blink, however, wide intra-individual and inter-individual variation in blink frequencies made simple differences difficult to compare. In this case, we calculated relative responses in blink frequency by dividing a subject's high dose caprolactam eye blink frequency by their control blink frequency to yield a measure of relative response to caprolactam. Comparing relative blink increase (manual method) with absolute eye irritation increase at 1 hour of exposure, we found that responses are correlated (Spearman rho coefficient=0.54, $p=0.01$).

OEHHA used the same approach in applying the Spearman rho test for correlation between eye irritation score and odor score. Because the odor of caprolactam was apparent to the participants the subjective eye irritation score may be influenced by the odor, similar to what has been observed for other airborne chemicals with unpleasant odors (Dalton, 2003). As with eye irritation, odor response was calculated by subtracting control odor score from high dose

odor score. At 1 hour of exposure, no correlation was found for absolute eye irritation score vs. absolute odor score (Spearman rho coefficient=0.04, *p*=0.86). Finally, the Spearman rho test was also applied to relative eye blink frequency vs. absolute odor score at 1 hour of exposure. No correlation was observed (Spearman's rho coefficient=0.20, *p*=0.41). These results indicate that eye blink responses and eye irritation responses are correlated, but neither is correlated with odor.

No correlation was apparent between eye blink response and eye redness response (Spearman's rho =0.38, *p*=0.10), which was also calculated by taking the difference of an individual's high dose and control redness scores. However, this is not inconsistent with reports by other investigators. For example, Lang et al. (2008) observed eye redness at the same level of formaldehyde exposure that caused increased eye blink frequency in one exposure scenario (0.5 ppm formaldehyde with 1.0 ppm peaks), but did not observe eye redness in a similar scenario that caused increased eye blink rate (0.5 ppm formaldehyde with 1.0 ppm peaks + ethyl acetate as an odor masking agent). These data suggest eye blink frequency is a more consistent and sensitive indicator of eye irritation than eye redness.

Case Reports and Human Sensitization Studies

We identified three peer-reviewed published case reports of workers heavily exposed to caprolactam developing seizures and other severe symptoms. The exposure levels in these reports were not quantified, however the descriptions suggest that the exposures could be considerably higher than typical occupational exposures.

Tuma et al. (1981) reported the case of a 22-year-old man who developed dermatitis of the hands and feet, nausea and vomiting, leukocytosis, and "grand mal" (i.e., generalized tonic-clonic) seizures three days after being transferred to a section of a plastics plant that involved caprolactam use. The caprolactam dust coated his clothing and exposed areas of skin when he arrived at the hospital. Dermal inflammation was also noted on the buttocks and thighs. The authors did not indicate that any respiratory distress was present; a chest roentgenogram was reported to be normal. After a few days of observation in the hospital, the skin lesions manifested desquamation (peeling) and erythema (redness and swelling), although the other symptoms had cleared. A comprehensive neurological investigation identified no underlying organic CNS abnormalities, consistent with the seizures having been a consequence of the work-related caprolactam exposure.

In a second case report from South Korea translated from Korean by OEHHA, two young men were hospitalized with nausea, vomiting, dermatitis on the hands and feet, and tonic-clonic seizures following occupational exposure to caprolactam (Woo et al., 1998). The men had been packaging caprolactam, one for two days

and the other for four days, working inside a caprolactam containment vessel. Laboratory testing documented leukocytosis and hyperglycemia. Brain CT scanning and EEG testing were reportedly normal. No further symptoms or seizures were seen over the two months after exposure. The authors concluded that the skin lesions and unexplained generalized tonic-clonic seizures in the men strongly indicated a causal effect of caprolactam intoxication.

In a third case report translated from Chinese by OEHHA, three workers in a Chinese plant were taken to a hospital emergency room with symptoms of dizziness, nausea and vomiting following handling or moving plastic bags containing chemical raw materials including caprolactam (Chen, 2002). Two of the workers (both of whom were working shirt-less) displayed tonic-clonic seizures, opisthotonus, froth around the mouth, upward-turned eyeballs, and a post-ictal altered mental status. There was no mention of any respiratory or dermal symptoms in the exposed workers. Physiological and hematological exams were generally normal. Urine caprolactam concentrations were stated to be 2.9-3.7 g/L, and 13.6-15.4 g of caprolactam was leached from the workers' clothing. Data on caprolactam metabolism indicate that only about 2% is excreted unmetabolized in urine. Although metabolism could be saturated with high exposure, the g/L amounts in the urine do not seem biologically plausible. More likely, the reported units of measure are incorrect. The colorimetric method used to estimate the urine caprolactam concentration appears to use mg/L as the unit of measure (Zhou, 1976), which would be a more reasonable caprolactam urine concentration in the workers in this case report.

Chen (2002) noted the two patients recovered and were released after a four-day hospitalization. It had been an extremely hot day (heat stroke was ruled out clinically) and the authors speculated that sweat facilitated the dermal absorption of caprolactam, particularly in the two workers that were shirt-less. The workers had a previous caprolactam exposure without reported symptoms.

These case reports suggest that neurotoxicity can be an important endpoint in humans heavily exposed to caprolactam. Moreover, dermal absorption can be an important exposure pathway and may also lead to dermatitis. Relevant to these human case reports, studies in rabbits show that concentrated caprolactam (50% aqueous caprolactam solution) placed on the skin can cause local irritation and be absorbed, leading to convulsions and death (Haskell Laboratory, 1950). Other animal studies with different exposure routes have also observed caprolactam-caused seizures (Goldblatt 1954; Gross 1984, reviewing the Eastern European literature). These animal studies will be discussed in greater detail in Section 5.3 to follow.

A review by Gross (1984) of the eastern European occupational studies conducted in the 1960s and 1970s suggests a significant number of workers may develop "hypersensitivity" to caprolactam. However, the methodology was not adequately described in these studies and there was co-exposure to other

chemicals. Exposure studies conducted in the West, some of which were not peer-reviewed, indicated caprolactam solutions of 1-5% did not cause skin irritation or act as a skin sensitizing agent.

Goldblatt et al. (1954) applied a 5% aqueous caprolactam solution to the skin of the inner forearms of six normal persons (4 men, 2 women) as a patch test left in contact for 48 hours. Goldblatt et al. also applied a 5% caprolactam solution in either alcohol or olive oil to the same area on volunteers and allowed to dry. The process was repeated daily for four days. In all cases, no irritant effects were produced. The authors concluded that caprolactam is not a skin irritant following these short-term exposures, and no evidence was found that it could act as a sensitizing or dermatitis agent.

In a study carried out in Haskell Laboratory in 1941 that was not published in a peer-reviewed journal, three human volunteers had a 1% aqueous solution of caprolactam applied to the skin (Haskell Laboratory, 1950). No skin irritation was reportedly produced. No other methods or descriptive information was provided.

In another non-peer-reviewed study (i.e., not published in a peer-reviewed journal) conducted in 1952-53 and recently reported to the U.S. EPA (2009a), a patch test was conducted in 204 human subjects to determine whether or not Nylon-6 containing 3-5% water-extractable caprolactam and dimers would produce primary skin irritation and/or sensitization in occupational exposures. No primary irritation or allergic sensitization was observed in the tested subjects. No other methods or descriptive information was provided. Animal data related to the question of sensitization from this study is summarized in Section 5.3.

In contrast to these negative human experimental studies there have been case reports consistent with caprolactam-related contact dermatitis. A worker with 29 years of experience in a Nylon-6 factory presented with an 18-month history of dermatitis (Aguirre et al., 1995). Patch testing with a 5% aqueous solution of caprolactam was positive for contact dermatitis. The lesions completely resolved following 2-month leave from work. In another case report, a woman developed dermatitis at a skin site where blue polyamide-6 suture thread had been removed following 10 days of placement following a dermatological procedure (Hausen, 2003). She had already undergone more than 40 similar procedures in the past. Patch testing was positive for caprolactam and the blue dye (acid blue 158) used in the thread. The patient, who was a hairdresser for 17 years, also had positive testing for ammonium persulfate and 2,5-diaminotoluene, two chemicals she was exposed to occupationally.

Occupational Exposure Limit Values for Caprolactam

The National Institute for Occupational Safety and Health (NIOSH) and the American Conference of Governmental Industrial Hygienists (ACGIH) currently have recommended occupational exposure limits for caprolactam (Table 4). The

ACGIH (2003) recommends summing both aerosol and vapor forms of caprolactam together to determine the total airborne concentration. NIOSH has two caprolactam recommendations, one for the 'dust' (NIOSH, 1995a) to describe the solid or particulate form of caprolactam that may cause dermal irritation and another for the "vapor" (NIOSH, 1995b) that refers to both airborne aerosol and vapor forms. Both sets of recommended limit values by NIOSH and ACGIH are influenced by the published findings by Ferguson and Wheeler (1973) that reported no irritation of any kind, or any other signs and symptoms of discomfort and malaise, in workers below 7 ppm (32 mg/m^3).

Table 4. Summary of the ACGIH and NIOSH limit values for caprolactam

Agency	Occupational Exposure Value
ACGIH	TLV-TWA, 5 mg/m^3, as inhalable aerosol and vapor
ACGIH	TLV-STEL, no specific data on which to base a TLV-STEL
NIOSH	REL-TWA, 1 mg/m^3, dust
NIOSH	REL-TWA, 0.22 ppm (1 mg/m^3), vapor
NIOSH	REL-STEL, 3 mg/m^3, dust
NIOSH	REL-STEL, 0.66 ppm (3 mg/m^3), vapor

The ACGIH (2003) has recommended a combined caprolactam aerosol and vapor threshold limit value (TLV) of 5 mg/m^3 (1.08 ppm) as a time-weight average (TWA) for a normal 8-hr workday and a 40-hr workweek. The ACGIH (2003) did not find that sufficient data were available to recommend skin or sensitization notations.

NIOSH (1995b) has established a recommended exposure limit (REL) for caprolactam vapor of 0.22 ppm (1 mg/m^3) as a TWA for up to a 10-hr workday and a 40-hr workweek, and 0.66 ppm (3 mg/m^3) as a short-term exposure limit (STEL). These lower recommended values, compared to ACGIH values, are intended to prevent early signs of irritation in some workers. Based on available human exposure responses, primarily Ferguson and Wheeler (1973), NIOSH felt a sufficient margin of safety was warranted to prevent such outcomes due to caprolactam vapor.

For caprolactam dust, NIOSH (1995a) recommended an REL value of 1 mg/m^3, and a STEL of 3 mg/m^3. These exposure levels appear to be largely based on an unpublished letter to the ACGIH TLV committee in 1972 when occupational limits were being determined by that body for caprolactam (Ferguson and Wheeler, 1973; ACGIH, 2001). In this letter, airborne caprolactam dust was reported to be irritating to the skin of some individuals at 5 mg/m^3, but adequate protection was provided by a limit of 1 mg/m^3 particularly when this was combined with respirator use. From this description, it may be inferred that the exposure limits for caprolactam as airborne dust were designed to lead to lower deposition onto surfaces that facilitate direct dermal contact and dermal irritation. As stated previously, however, airborne vapors are likely to lead to surface condensation as well.

The U.S. Occupational Safety and Health Administration (OSHA) has promulgated a permissible exposure limit (PEL) neither for caprolactam vapor nor caprolactam dust. Cal/OSHA, however, in 1973 promulgated a PEL of 1 mg/m^3 and STEL of 3 mg/m^3 for caprolactam dust (Cal/OSHA, 2011). For the vapor form, the Cal/OSHA PEL is 5 ppm (20 mg/m^3) and the STEL, 10 ppm (40 mg/m^3). These occupational limit values were likely adopted from earlier ACGIH (2001) limit values for caprolactam before they were revised by the ACGIH in 2003.

The ACGIH (2001) originally had higher exposure limits that mirrored Ferguson and Wheeler's conclusion that a worker threshold value of 5 ppm (23 mg/m^3) is recommended based on the absence of reported distress among those working at concentrations up to 7 ppm. The subsequent reduction of the ACGIH (2003) exposure limit to well below this worker threshold value suggests that that organization no longer accepted the findings by Ferguson and Wheeler (1973) in this regard.

5.2 Acute Toxicity to Infants and Children

No studies were located regarding acute toxicity to infants and children exposed to caprolactam. We found no studies of inhalation exposure to young or pregnant animals that could shed insight into acute toxicity in infants and children. In pregnant mice, oral delivery of radiolabeled caprolactam was rapidly absorbed from the stomach and freely distributed into all tissues, including the fetuses (Waddell et al., 1984). Some residual radioactivity was noted in the umbilical cord, amnion, and yolk sac. No radioactivity was retained in any other fetal tissues.

5.3 Acute Toxicity to Experimental Animals

Relatively few peer-reviewed studies of acute caprolactam exposure in experimental animals have been conducted. Acute inhalation, oral, and parenteral exposure studies are summarized below, including some non-peer-reviewed studies, to provide the full spectrum of effects resulting from acute intoxication from caprolactam exposure. Due to caprolactam's respiratory irritant action, dermal and inhalation sensitization studies are also reviewed. None of the sensitization studies was peer-reviewed. A summary of the animal toxicity findings, including acute and multi-day exposures, is presented in Table 5 at the end of this section.

The BASF Chemical Company conducted unpublished[1] acute exposure studies in the 1960s and 70s that were reported by Ritz et al. (2002). In the rat, an oral LD_{50} of 1155 mg/kg is reported. Symptoms of acute intoxication were tonoclonic convulsions. In an acute toxicity study on rats and mice, the NTP (1982) administered caprolactam in corn oil by gavage to groups of five males and five

[1] unpublished means not published in a peer reviewed journal

females. The LD_{50} for male and female mice were 2070 and 2490 mg/kg, respectively. The LD_{50} for male and female rats were 1650 and 1210 mg/kg, respectively. No signs or symptoms of toxicity were discussed.

Goldblatt et al. (1954) observed 66% mortality in rats injected intraperitoneally with 800 mg/kg caprolactam with the appearance of delayed spasms. Lower non-fatal doses (500-600 mg/kg) resulted in tremors, apprehension, depression of temperature, and occasional chromodacryorrhea. Concentrations of 900 mg/kg and above proved fatal and resulted in epileptiform convulsions, salivation, and bleeding from the nares. Goldblatt et al. (1954) also injected rabbits intravenously with non-fatal doses of caprolactam ranging from 100 to 300 mg/kg. The effects were severe including mydriasis, salivation, accelerated respiration, tremors, opisthotonic-like muscle contractions, and convulsions. The latter end-point has already been summarized previously in relation to human case reports of caprolactam-associated seizures.

Similar results were observed in the foreign toxicology literature (published mainly in Russian and German) of the 1950s and 1960s and reported in a review by Gross (1984). Caprolactam LD_{50} studies in experimental animals and exposure to high doses of caprolactam by intravenous and intraperitoneal injection produced tremors, epileptiform convulsions, salivation and bleeding from the nostrils. In an unpublished[1] study by Haskell Laboratory (1950), an approximate lethal dose of 3375 mg/kg was observed in rats administered by gavage. Rats receiving 1500 mg/kg developed convulsions and some showed slight bleeding from the nose and mouth.

In an unpublished[1] industrial study, four-hour exposure of rats to 5,250, 8,350, or 10,120 mg/m^3 caprolactam aerosol via a head-nose inhalation system resulted in eyelid closure, shallow to spasmodic breathing, and mild to strong defense reactions (BASF, 1985). After exposure, steppage gait, bloody nasal secretions, spasmodic breathing, marked tremor, and bloody lacrimation were observed. LC_{50}s of 9,600 and 7,080 mg/m^3 were recorded for male and female rats, respectively. In rats that died, general circulatory congestion, elevated hyperemia of the lung, moderate to severe fatty degeneration of the liver, and ischemic tubular nephrosis in the renal cortex were found. No additional deaths occurred after one day post-exposure and all surviving animals appeared normal 3 days post-exposure. Histopathological examination of the organs in surviving rats 14 days post-exposure was described as “unremarkable”.

In another unpublished[1] study, two rats exposed to a nominal particle caprolactam concentration expressed as 14,000 ppm (sic) for 17 min showed signs of general discomfort and inflammation around the eyes and nose (Haskell Laboratory, 1950). Note that particle exposures should be expressed in mg/m^3, which in this

[1] unpublished means not published in a peer reviewed journal

case would be approximately 65,000 mg/m^3. No gross pathology or micropathology was detected at sacrifice following a nine-day observation period.

The U. S. Consumer Product Safety Commission contracted a study of sensory and pulmonary irritation in Swiss-Webster mice exposed to compounds emitted from carpet and carpet-related products, including caprolactam (CPSC, 1996). The animals were placed in a head-only glass plethysmograph and exposed to 13.5 mg/m^3 caprolactam vapor, the highest attainable exposure concentration. The study protocol called for a one hour exposure, followed by a recovery period of 15 minutes in clean air, then exposure to the same concentration of caprolactam for another hour.

Sensory irritation was defined by a 12% or greater group decrease in the mean respiratory frequency, the minimum level of respiratory depression needed to classify an exposure as having a positive sensory irritation response (CPSC, 1996). By this approach, no measurable sensory irritation or reduction in respiratory rate was observed in the mice during the caprolactam exposure. However, the CPSC (1996) notes that measurable respiratory irritation in mice using this method usually occurs at levels 10 to 100 times higher than levels which would result in irritation in humans.

Inhalation and Dermal Sensitization/Irritation Studies

We consider sensitization here under acute exposure effects because the anamnestic response is manifested with an acute re-challenge, even though the process of sensitization itself may require repeated subacute or even chronic exposures.

In a skin absorption study, a 50% aqueous solution of caprolactam was applied to a shaved area between the shoulder blades of rabbits (Haskell Laboratory, 1950). The approximate lethal dose was 3375 mg/kg producing pathology similar to hypovolemic shock. Clinical observations included tremors, convulsions, and bleeding from the mouth and nose analogous to those observed in rats receiving oral doses. Edema and congestion of the skin at the site of application was noted, which may have increased dermal absorption as a result of skin damage. This study was not published in a peer-reviewed journal.

Gross (1984) reviewed the eastern European literature conducted in the 1970s concerning dermal sensitization studies in animals. It was claimed in these studies that both intracutaneous and dermal application of caprolactam in guinea pigs resulted in “sensitization.” In the case of intracutaneous injections, the development of contact dermatitis was interpreted as indicative of successful sensitization. In one of two cases, it was claimed guinea pigs became sensitized to caprolactam by inhalation. However, other studies described below could not reproduce assertions of inhalation sensitization.

In an unpublished[1] report submitted to U. S. EPA, groups of four male albino guinea pigs were exposed for 30 min on 5 consecutive days to 3, 10, or 30 mg/m^3 aerosols (1.5 micron) generated from a 15% aqueous caprolactam solution (U. S. EPA, 1994b; Rinehart et al., 1997). On day 19, 26, 33 and 40, animals were challenged for 30 min with 30 mg/m^3 caprolactam. Animals were monitored with whole-body plethysmography for indications of irritation and coughing, and pulmonary hypersensitivity was monitored using respiratory frequency, tidal volume, and airway constriction as criteria for effect. Caprolactam failed to induce immediate or delayed pulmonary hypersensitivity with this protocol, which has been positive for ovalbumin and trimellitic anhydride. In addition, there was no evidence of respiratory tract irritation at any exposure concentration.

In unpublished[1] work carried out by the BASF Chemical Company, guinea pigs were exposed to repeated epicutaneous application (50% ether solution; 10 times) or intracutaneous injection (0.1% in physiological NaCl solution) (Ritz et al., 2002). Neither treatment caused local irritation or sensitization to the skin.

In an unpublished[1] study carried out in 1941, a skin irritation test with a 66% aqueous solution of caprolactam was conducted in 10 albino guinea pigs (Haskell Laboratory, 1950). Initial application of the aqueous caprolactam solution to unbroken shaved skin resulted in erythema in one animal, faint erythema in two other animals, and negative results in the remaining 7 animals. The researchers concluded caprolactam produced only mild dermal irritation in the guinea pigs.

To further test for sensitization, a maximization test was conducted that consisted of a series of 6 treatments of a 66% aqueous solution of caprolactam to broken skin, or 6 intradermal injections of 0.1 ml of a 0.1% aqueous solution (Haskell Laboratory, 1950). This was followed by a rest period of two weeks, and then the 66% aqueous caprolactam solution was again applied to the unbroken skin at the same site as the original application. Seven of ten animals manifested dermal reactions indicating that sensitization had occurred. A final intradermal injection and application to broken skin likewise showed an increase in intensity of the reaction consistent with sensitization. Although the sensitization potential is limited by using an irritant concentration for challenge treatment, the researchers considered caprolactam should be considered a mild sensitizer on the basis of the strength of the reaction they observed.

In a similar (unpublished) maximization test protocol, 20 female guinea pigs received intradermal application of caprolactam (3.0% w/v) in water, or topical application of caprolactam (75% w/v) in water (Springborn Laboratories Inc., 1991). Challenge responses in the induced animals were compared to those of the controls. Blood samples were obtained prior to study initiation and following the challenge for evaluation of standard hematology parameters. Additionally,

[1] unpublished means not published in a peer reviewed journal

plasma histamine was determined for selected test and control animals following challenge. Based on the concurrent mild dermal reaction in the control group animals and the fading of reactions from 24 to 48 hours, caprolactam was not considered to be a contact sensitizer in that study.

In a Buehler test in rabbits, induction test animals were patched with 25% w/v caprolactam in water 3 times within 3 weeks (Springborn Laboratories Inc., 1991). In the challenge phase, the test group animals received 25% w/v caprolactam in water in a patch. Ten animals each were used in the challenge control and the rechallenge control groups. Dermal reaction was scored 24 and 48 hours after removal of the patch. Minimal dermal reaction was observed in both the test animals and negative control animals after the challenge as well as after the rechallenge. Mean dermal scores were also comparable between both groups. The skin sensitization potential of caprolactam was limited by using an irritant concentration for the challenge treatment. Therefore, caprolactam was not considered to be a contact sensitizer under the test conditions chosen. This study was also not published in a peer-reviewed journal.

In a dermal sensitization test by Rinehart et al. (1997), groups of 20 female albino guinea pigs were tested with 25% aqueous caprolactam solution using either the traditional modified Buehler or maximization test designs. Groups of 5 guinea pigs were treated with 5% DNCB (probably 1-Chloro-2,4-Dinitrobenzene) as a positive control. After the second challenge dose had been evaluated, blood samples were obtained for measurements of leukocytes, differential counts and plasma histamine levels. Neither test regimen showed positive results for animals treated with caprolactam. There were no body weight changes or any effects on hematologic components or plasma histamine levels caused by treatment with caprolactam. This study was reported in the journal only in abstract form.

A report of a skin sensitization test conducted on up to 6 guinea pigs and 4 dogs at the end of a subchronic inhalation exposure regimen was submitted to U. S. EPA (U. S. EPA, 2009a). This study (not published in a peer-reviewed journal) was conducted in 1952-53 and only recently reported to the U.S. EPA. All animals were exposed to 444 mg/m^3 caprolactam as a fume (i.e., a solid suspension generated by heating caprolactam in air) 6 hrs/day for 43 exposures. Half of the guinea pigs and 3 of the dogs were then exposed to 1020 mg/m^3 on exposures 44 through 67 or 73. Observations were made one-hour, 24-hours, and 48 hours after patch application. Both guinea pigs and dogs acquired skin sensitization. No descriptive information was provided that clarify the severity of the response (although it was reported to be “mild”).

In summary, acute caprolactam exposure in animals produced severe neurological effects. Caprolactam given orally by gavage, injected intravenously or intraperitoneally, or applied to the skin, can cause convulsions. Inhalation studies at lethal or near-lethal concentrations resulted in severe tremors. Dermal and inhalation sensitization test were mostly negative. Dermal sensitization has

been noted in some studies, however, although interpretation of these is complicated by dermal irritation effects. A concern with the overall acute data is that most of these reports were not published in a peer-reviewed journal, and results were often insufficiently reported or published.

Table 5. Effects of Caprolactam Exposure in Experimental Animals

Species	Exposure	Response	Reference
Inhalation Studies (Detailed summaries in Section 5.3 and 6.3)			
Rats	65,000 mg/m^3, nominal exposure concentration, 17 min	Signs of general discomfort, eye and nose inflammation	Haskell Laboratory, 1950
Mice	13.5 mg/m^3, 2 hrs	RD_{50} study No measurable sensory irritation or reduction in respiratory rate	CPSC, 1996
Rats	0, 5250, 8350, 10,120 mg/m^3 for 4 hrs	LC_{50} = 9600 mg/m^3 (males) LC_{50} = 7080 mg/m^3 (females) Eyelid closure, spasmodic breathing, steppage gait, marked tremor, bloody eye and nasal secretions	BASF, 1985
Rats	Nominal exposure to 13,900 mg/m^3 for 2 hrs, then 1-2 hr exposures to 12,500 to 31,500 mg/m^3 on 5 successive days	General discomfort, eye and nasal inflammation during exposure. Slight lung edema and spleen congestion 3 days after exposure	Haskell Laboratory, 1950
Guinea pigs	0, 3, 10, 30 mg/m^3 30 min/day for 5 days Challenge on day 19, 26, 33 and 40 with 30 mg/m^3 for 30 min	No indication of sensory irritation, coughing or pulmonary hypersensitivity as measured by whole body plethysmography	Rinehart et al., 1997
Guinea pigs	118-261 mg/m^3 for 7 hr/day for 7 days	Observed for irritant effects: Occasional cough seen	Goldblatt et al., (1954)
Guinea pigs	51 mg/m^3 5-8 hr/day for 26-30 days	Slight irritation of nasal and tracheal mucosa	Hohensee et al. (1951)

Species	Exposure	Response	Reference
Dogs, guinea pigs, rats, rabbits	444 mg/m^3 6 hrs/day for 43 exposures, then 1020 mg/m^3 6 hr/day for 23 to 29 more exposures	Dogs: aggravated sores and eyes. Low blood pressure, tremors, weakness, coughing, dense froth around mouth at 1020 mg/m^3. Rabbits: slight corneal damage and eye irritation. Rats: no specific toxic findings Guinea pigs: nephritis	Conducted in 1952-53. Summarized in U.S. EPA, 2009a
Rats	0, 24, 70, 243 mg/m^3 5 days/wk, 13 wks	Treatment-related red facial stains, clear nasal discharge, moist rales, labored breathing. Nasal respiratory and olfactory mucosal lesions, and laryngeal tissue lesions.	Reinhold et al., 1998
Oral Gavage Studies (Detailed summaries in Section 5.3)			
Rats	No exposure dose information provided	LD_{50} = 1155 mg/kg Tonic-clonic convulsions	Summarized in Ritz et al., 2002
Rats & mice	Rats: 681, 1000, 1470, 2150 & 3160 mg/kg Mice: 1000, 1470, 2150, 3160 & 4640 mg/kg	Male rat LD_{50} = 1650 mg/kg Female rat LD_{50} = 1210 mg/kg Male mice LD_{50} = 2070 mg/kg Female mice LD_{50} = 2490 mg/kg Symptoms not described	NTP, 1982
Rats	Detailed exposure dose information not provided	LD_{50} = 3375 mg/kg 1500 mg/kg resulted in convulsions, bleeding from mouth and nose	Haskell Laboratory, 1950
Dermal Toxicity and Sensitization Studies (Detailed summaries in Section 5.3)			
Rabbit	50% aqueous solution of caprolactam applied to shaved area of skin	LD_{50} = 3375 mg/kg Tremors, convulsions, bleeding from mouth and nose, skin damage at site of application	Haskell Laboratory, 1950
Guinea pigs	10 epicutaneous applications of 50% aqueous solution, or 10 intracutaneous injection of 0.1% aqueous solution	Neither treatment caused local irritation or sensitization to the skin	Summarized in Ritz et al., 2002

Species	Exposure	Response	Reference
Guinea pigs	Maximization test with 6 skin applications of 66% aqueous solution, or 6 intradermal injections of 0.1% aqueous solution	Initial application produced erythema in some animals. Re-exposure of caprolactam by both methods after 2 wk rest period resulted in mild sensitization	Haskell Laboratory, 1950
Guinea pigs	Maximization test with 75% aqueous caprolactam solution	Dermal reaction in control animals. Challenge application produced no sensitization or increased plasma histamine	Springborn Laboratories Inc., 1991
Rabbits	Buehler patch test with 25% aqueous caprolactam solution	Comparable minimal dermal reaction in test and negative control animals after challenge and rechallenge	Springborn Laboratories Inc., 1991
Guinea pigs	Buehler and maximization tests with 25% aqueous caprolactam solution	After challenge and rechallenge, no positive results for sensitization, or change in body weight, plasma histamine, leukocytes or differential counts	Rinehart et al., 1997
Guinea pigs & dogs	Skin patch test following 43 exposures at 444 mg/m^3, and 23 to 29 additional exposure at 1020 mg/m^3	Observations made at one hour and 24 and 48 hours after patch application. Mild skin sensitization was observed. Caprolactam patch concentration not stated.	US EPA (2009)_ [an 8(a) submission to US EPA]

6. Chronic Toxicity of Caprolactam

6.1 Chronic Toxicity to Adult Humans

Occupational exposure to caprolactam is known to cause dermal, eye and upper respiratory tract irritation with acute or recurrent acute exposure, but occupational studies with prolonged caprolactam exposure in workers were considered by OEHHA to be inadequate for use as the basis of a chronic REL.

Gross (1984) summarized the early foreign literature regarding industrial exposure to caprolactam. With a few exceptions, the pertinent publications were Russian. These reports describe diverse complaints and abnormalities of the various organ systems in people exposed in factories producing nylon. The exposures in no instance were only to caprolactam. Exposure to caprolactam was commonly associated with exposure to dinyl oxides, such as diphenyl oxide. Other

chemicals often associated with caprolactam exposures were cyclohexane, cyclohexanol, cyclohexanone, benzene, acetone, and trichloroethylene.

In a report from Germany translated from German by OEHHA, end of shift complaints by workers exposed to caprolactam at a factory included irritability, nervousness, heartburn, bloating, nose bleeds, upper airway inflammation, and dry and chapped lips and noses (Hohensee, 1951). Exposure included both the vapor and crystal, or dust, forms of caprolactam. Headache in response to the odor and unpleasant taste of the caprolactam vapor was also reported. All these symptoms subsided after a short (but unspecified duration) stay in fresh air. Factory inspection of the caprolactam concentration in the spinning room revealed a concentration of 61 mg/m^3, while the concentration in the laboratory room was 16-17 mg/m^3.

Although Ferguson and Wheeler (1973) were primarily focused on acute effects of airborne caprolactam exposure, the researchers also took 8-hr time-weighted average (TWA) measurements at two facilities and reviewed medical records. Other than dermal injuries resulting from direct contact to concentrated caprolactam solutions, no general health problems requiring medical follow-up were found in a review of medical records collected during the 18 years of plant operation. In addition, no worker had been removed or asked to be removed from exposure to caprolactam vapor for health reasons during plant operation.

At the caprolactam polymer plant, approximate 8-hr time-weighted average (TWA) air samples were collected from various locations in a work area over five days (Ferguson and Wheeler, 1973). The 8-hr TWA air concentrations of caprolactam vapor during working hours were 3.2 ppm (14.8 mg/m^3) with a range of 1.3 to 6.9 ppm (6.0 to 31.9 mg/m^3) at location 1, and 1.1 ppm with a range of <0.5 to 4.5 ppm (<2.3 to 20.8 mg/m^3) at location 2. Based on the percent time worked in specific locations of the caprolactam-contaminated rooms, the worker exposure durations were estimated to be about 15 to 45 min at location 1, and 1 to 4 hrs at location 2. At the caprolactam monomer plant, 8-hr TWA caprolactam vapor concentrations at various sites over a 3-week period were collected. The concentration of caprolactam sampled at various worksite locations ranged from 0.2 to 17.6 ppm (0.9 to 81.5 mg/m^3). Worker exposure durations in the caprolactam-contaminated areas ranged from 10 min to almost 3 hrs.

From the 8-hr TWA data collected, Ferguson and Wheeler (1973) concluded that working atmospheric concentrations up to about 7 ppm (32 mg/m^3) at the caprolactam polymer plant generally resulted in no reported distress of interviewed workers in active and semi-active areas. This data supported their estimate of a worker irritant response threshold of 5 ppm (23 mg/m^3) based on the acute exposure portion of their study.

There are significant deficiencies in the Ferguson and Wheeler report that prevent it from use as the basis of an OEHHA chronic REL. As also noted by the U.S.

EPA RfD/RfC Work Group, significant deficiencies included lack of information on the number of workers and the average duration and distribution of exposure (U. S. EPA, 1994b). Also, no historical air levels are given, all exposures are determined from area rather than personal samplers, and no attempt was made to reconstruct individual exposure histories.

Kelman (1986) conducted a clinical and occupational history of eight workers, seven of which were smokers, at a Nylon-6 manufacturing plant. Several of the workers (number not given) had complained of “some degree” of eye, nose, and throat irritation, although it was unclear from the study if the irritation was chronic in nature. All but one reported peeling of the skin on the hands. Five workers showed abnormal maximal expiratory flow volumes. The author considered the lung function tests unremarkable when the smoking history of the workers was taken into account. Blood and urine samples were collected for assessment of hematological, hepatic and renal functions. No evidence of systemic toxicity was found.

Exposure by Kelman (1986) was described as caprolactam vapor from heat-curing ovens, which subsequently condensed into a fume in the workplace air. Contact of the fume with cooler surfaces resulted in the formation of light feathery flakes. Average worker exposure was 4.8 years (range 9 months to 13 years) and mean atmospheric caprolactam dust concentrations at the time of the study were 84 mg/m^3 (range: 22-168 mg/m^3) for static samplers and 68 mg/m^3 (range: 6-131 mg/m^3) for personal samplers. Recovery of caprolactam vapor from distilled-water bubblers was considered negligible, which the authors interpreted as indicating exposure was limited to caprolactam dust. The caprolactam dose and exposure durations for individual workers were not provided in this study, and a characterization of the caprolactam particle sizes was not performed. The reference to formation of “light feathery flakes” suggests that some part of the caprolactam was in particle sizes too large to be inhaled and may not be relevant for inhalation exposure.

Billmaier et al. (1992) conducted an industrial exposure study of selected workers in two caprolactam plants, Chesterfield and Hopewell. Forty-nine workers were selected (27 smokers/ex-smokers) with 63 controls (workers not working in caprolactam areas, 42 smokers). The controls were matched to the exposed workers (all males) for age, race and smoking status. The workers selected had an average work exposure of 18.7 years (range: 8.2-31.7 years) against matched controls. The level of caprolactam in the work areas was determined mainly by personnel monitoring. The monitoring method detected total caprolactam and did not differentiate between various states of the material. In operations where caprolactam or the polymer is heated and/or wet or in water solution, the airborne caprolactam was assumed to be in the vapor state [OEHHA notes that this description suggests much of the caprolactam may also be in aerosol form, especially at concentrations above the saturated vapor concentration of 13 mg/m^3]. The average concentrations from occasional monitoring over the

previous 10 years at the Chesterfield plant averaged 4.5 mg/m^3 in the Polymer 25 area and 9.9 mg/m^3 in the Spinning 26 area (area monitoring only). Short term measurements of 15-59 minutes during specific plant operations that represented maximum short-term exposures to caprolactam ranged up to 34.8 mg/m^3. For the Hopewell plant, the levels were 4.2-7.8 mg/m^3 from occasional monitoring over 10 years, and an average of 17 mg/m^3 with a range of 2.3-30.8 mg/m^3 from short term measurements.

Pulmonary function tests were obtained by Billmaier et al. (1992) from all exposed and control workers. Pulmonary function tests began in 1978. "Nurses notes" used were from Chesterfield workers. These notes were obtained from workers who were ill, injured, had a physical examination or a return to work examination, or others over a period of 11 years. Only a few episodes of injury or illness were noted in the medical records that were specifically related to caprolactam exposure. One employee reported dermatitis on two separate occasions, and another employee reported dermal irritation following direct exposure to a lactam-containing solution. A third employee complained of eye irritation on one occasion and reportedly inhaled partially polymerized nylon flakes on another occasion, leading to nausea. No specific caprolactam exposure-related nose or throat symptomatology was reported. However, "symptoms" recorded in the notes may not have been assessed as this was optional.

There were no significant differences between exposed workers and their controls in the pulmonary function tests or lung function over the years (Billmaier et al., 1992). Wide differences were shown in the initial (using a Collins Eagle spirometer from 1980 to 1988) and last (using a Puritan Bennet spirometer which replaced the Collins Eagle spirometer) FEV_1/FVC ratios between smokers (n=21), ex-smokers (n=12) and non-smokers (n=7) but not between smokers and controls. The measurement of FEV_1/FVC ratios is sensitive to changes in lower airway function. The authors concluded that there would be differences in the FEV_1/FVC ratios between the exposed workers and their controls if they were present.

OEHHA notes several uncertainties with Billmaier et al. (1992) that preclude it from use as the basis of a chronic REL. Differences in the FEV_1/FVC ratios in smokers, ex-smokers and non-smokers may be due to the fact that tobacco smoke is inhaled deeply and reaches the lower airways. Caprolactam vapor may not be inhaled as deeply because it is a water soluble gas and will primarily deposit in the upper airways. Other toxicological studies summarized in this document indicate the endpoint for caprolactam exposure is the upper respiratory tract. Thus, FEV_1/FVC ratios may not be an effective measure of caprolactam effects. U.S. EPA (1994b) also notes that the spirometry performed was not in accordance with current guidelines and quality assurance procedures.

Another weakness in Billmaier et al. is that individual worker exposure histories could not be clearly determined due to high variability in caprolactam levels and

changes in job responsibilities throughout the workday. As noted earlier, the irritation data from "nurses' notes" are probably unreliable and were apparently not collected systematically for all workers. Finally, the authors did not conduct a survey of the workers regarding sensory irritation symptoms or examine the upper respiratory tract for signs of inflammation.

Occupational studies of caprolactam workers have been conducted in China and were translated from Chinese into English by OEHHA. An occupational study of the health effects of caprolactam was conducted in 154 exposed workers at a Chinese caprolactam production plant (Li, 1996). The mean age of the exposed workers (111 men, 43 women) was 36.0 years (18 to 56 years of age), and the average working time at the facility was 15.7 years (1 to 22 years). The exposure group was divided into Extraction Section workers and Steaming and Packaging Section workers for assessment of health effects. Another 91 workers in the same plant but with no history of exposure (58 men, 33 women) was the control group. Their mean age was 38.1 years (17-55 years of age), and an average working time of 14.8 years (1 to 20 years).

Area air monitoring data for caprolactam over a ten year period from 1983 to 1992 were presented, with 19 to 28 samples collected per year for a total of 249 samples (Li, 1996). The concentration range over this time period was 0.5 to 110.0 mg/m^3 with a geometric mean of 9.2 mg/m^3. In the most recent year of sampling, 1992, the range was 0.6 to 6.5 mg/m^3 with a geometric mean of 2.0 mg/m^3. Statistically significant health effects and area air monitoring concentrations are presented in Table 6 for each work section of the facility. No air monitoring data were collected for the control group.

Table 6. Workplace caprolactam air concentrations and worker signs and symptoms of exposure.

Section	**Geometric Mean and Concentration Range (mg/m^3)**	**Significant Health Effects Compared to Control Group**
Extraction Section workers	11.8 (2.1 - 110.0) (n=92 air samples)	0.01<*p*<0.05: dizziness, insomnia, nosebleed, dermal lesions, reduced leukocytes P<0.01: nasal symptoms
Steaming Section workers	8.5 (0.5 – 98.6) (n=80 air samples)	0.01<*p*<0.05: nasal symptoms, dermal lesions, reduced leukocytes
Packaging Section workers	6.7 (0.5 – 38.6) (n=77 air samples)	

Health effects related to caprolactam exposure included dermal symptoms such as dry, smooth, cracked, scaling and peeling skin. Nasal symptoms included dryness, rhinitis and sinusitis. A reduction in leukocytes was observed, defined as <4.0x10^9/L. Li (1996) noted that workers that inhaled high concentrations of caprolactam experienced a sense of "tight chest". The author surmised that this

symptom was possibly due to laryngeal mucosal or tracheal/bronchial irritation resulting in contraction. Leukocyte classification, liver function, ECG, hemoglobin and urinalysis were considered normal in the exposed workers. The authors speculated that exposure to other chemicals in the factory did not have an impact on the health of the workers.

The occupational exposure study by Li (1996) provided a large cohort of exposed workers of sufficient exposure durations. However, the lack of personal air monitoring data make it problematic for OEHHA to establish a point of departure based on the geometric means presented. Historical air sampling for the previous 10 years is included in the paper, with the earlier years of sampling resulting in the highest exposures. Individual exposure histories including the earlier years of higher exposure would have been useful. Although the author indicated that co-exposure to other chemicals was not a concern, the caprolactam extraction process is known to include solvents such as benzene, toluene and chlorinated hydrocarbons (Ritz et al., 2002). Benzene is a known hematotoxic agent. The briefness of the report and the lack of a caprolactam air concentration for the control group are other deficiencies that prevent the study from use as the basis of a REL.

The health effects of caprolactam were investigated in workers at a different Chinese caprolactam production plant by Xu et al. (1997) (translated from Chinese by OEHHA). The mean age of the exposed workers (77 men, 48 women) was 29.3 years (20 to 57 years of age), and the average working time at the plant was 9.4 years (1 to 36 years). From the same plant, 120 workers (56 men, 64 women) with no history of poisoning or exposure to caprolactam dust were selected as the control group. Their mean age was 33.1 years (20 to 49 years of age), and an average working time of 12.6 years (1 to 28 years). The smoking rate for control males (55.36%) was slightly higher than the smoking rate for exposed males (43.06%). None of the women in the study smoked.

In the Xu et al., (1997) study, two air samples each were collected at four work stations with potential exposure to caprolactam. A dust sampler was used to collect caprolactam and measurement was by weighing the filter paper. The average air concentration of caprolactam at the work stations was 3.79 mg/m^3 (range: 0 to 7.93 mg/m^3). No air samples were collected in the control areas. Statistically significant ($p<0.05$) increases in insomnia, nausea and loss of appetite was reported by the exposed workers. Other questions (headache, dreams, stomach ache and back pain) were similar to controls.

Biochemical indicators of liver and kidney function and a peripheral blood micronucleus test were similar to control values (Xu et al., 1997). A peripheral blood lymphocyte chromosomal aberration test showed no difference from control values. However, exposed smokers showed a statistically significantly higher chromosomal aberration rate (2.50% vs. 1.36%, $P<0.05$) than smoking control group workers. No difference was seen between non-smoking exposed and

control workers. The authors suggested a synergistic action for higher chromosomal aberration rate may exist with smoking and caprolactam exposure. In females, a higher rate of dysmenorrhea (i.e., painful menstruation) was observed in exposed vs. controls (37.5% vs. 17.5%, $p<0.01$). No difference was seen between exposed and control groups regarding other menstrual disorders or pregnancy and delivery complications.

The study by Xu et al. (1997) did not ascertain sensory and dermal irritation, one of the most common complaints with industrial exposure to caprolactam in other studies. Air sampling collected particles (i.e., caprolactam dust), but not the vapor form of caprolactam that may have been present in the air. The dust sampler would reflect total airborne particulate matter, not just caprolactam. It was unclear from the report if the workers were exposed to other forms of particulate matter. The authors suggest some level of exposure to other chemicals used during the extraction process occurred, but was not measured. Historical air sampling data were not presented.

Another health study was conducted in a Chinese combined caprolactam production and Nylon-6 polymerization facility (Lan et al., 1998). In this report, the caprolactam concentration was purported to be below 5.6 mg/m^3 in each caprolactam work area, but how the air samples were collected and analyzed was not described nor was the mean and range of caprolactam concentrations presented. The authors reported statistically significant increases ($p<0.01$) in dizziness, headache, fatigue, insomnia, memory loss, loss of appetite, skin itching, and bleeding gums in the exposure group of 104 workers compared to a control group of 77 workers from a pharmaceutical factory. Dry nose was also present in the exposed group ($0.01<p<0.05$).

The workers in the Lan et al. (1998) study had an average work history of 4 years at the factory, and had an average age of 24.85 years. The control group had an average work history of 6 years and an average age of 30.20 years. Drinking and smoking histories were similar between the two groups. Other survey results, including liver function, blood tests, ECG, and chest x-ray, were normal in the exposed group. The authors indicated poor industrial hygiene among the workers likely resulted in both inhalation and dermal exposure to caprolactam.

Limited case reports of allergic contact dermatitis resulting from repeated exposure to caprolactam followed by an acute re-challenge response have been published. These have been summarized previously.

There are also data from a chronic oral human exposure protocol. In that study, investigating caprolactam as a weight-reducing agent, groups of obese patients received either placebo (n = 26), 3 g (n = 62) or 6 g (n = 28) of caprolactam daily as wafers or as tablets for 18 months (Riedl et al., 1963). The study participants were also instructed to eat a 1000-calorie weight-loss diet. The subjects receiving the placebo manifested no reduction in weight, while subjects treated with 3 and 6

gm caprolactam per day showed weight reductions averaging about 0.025 and 0.05 kg/day, respectively.

Those administered caprolactam showed minimal adverse effects other than weight loss. Of note, however, thirst was reported by one patient and a rash was observed in one patient. Factoring in body weights at the beginning of the study, average daily caprolactam intake of patients administered 3 g caprolactam daily was approximately 26 and 28 mg/kg body weight for males and females, respectively. The average daily intake of patients administered 6 g caprolactam was approximately 52 and 56 mg/kg body weight for males and females, respectively.

Riedl *et al*. (1963) also investigated the effects of caprolactam on intermediary metabolism when obese patients were administered 1 g glucose per kg body weight. Caprolactam treatment was not clearly specified, but appeared to also consist of 3 or 6 g doses per day for at least two months prior to glucose loading. Blood lactic acid levels were reduced in those patients receiving caprolactam. Blood sugar and levels of citric acid and non-esterified fatty acids in blood were unaffected by caprolactam treatment.

A summary of the human exposure findings discussed in this document is presented in Table 7 below.

Table 7. Effects of Caprolactam in Humans

Caprolactam Exposure	Response	Reference
Exposure chamber studies and occupational surveys (Detailed summaries in Section 5.1 and 6.1		
20 participants, controlled chamber exposures to 0, 0.15, 0.5, 5 mg/m^3 for 6 hrs	Positive trend for eye blink and irritation with increasing concentration; increased eye blink and irritation at 5 mg/m^3. Positive trend for odor annoyance with increasing concentration; increased odor annoyance at 0.15 mg/m^3	Ziegler et al. 2008
5 non-acclimated workers, 46, 65, 116, 482 mg/m^3 from uncontrolled source for several minutes	Transient nasal and throat irritation in most or all participants at all concentrations. Eye irritation in 1 or 5 participants at 482 mg/m^3	Ferguson & Wheeler, 1973
61 mg/m^3 in spinning room, 16-17 mg/m^3 in laboratory.	Irritability, nervousness, heartburn, bloating, headache, nose bleeds, upper airway inflammation, dry and chapped lips and noses, unpleasant taste in the mouth.	Hohensee, 1951
8 workers, 4.8 yr mean exposure to mean of 68 mg/m^3 at time of study with personal samplers	Complaints of eye, nose and throat irritation from some workers, 7 of 8 workers had dermatitis	Kelman, 1986
49 workers, 63 controls 18.7 yr work history, 4.5 mg/m^3 and 9.9 mg/m^3 in 2 areas by occasional monitoring over 10 years	Reliance on nurse's notes, no formal interviews of workers. 3 reports of dermatitis, 1 report of occasional eye irritation with nausea from inhalation of caprolactam flakes.	Billmaier et al. 1986
154 workers, 91 controls 15.7 yr work history, area sampling over 10 yrs in 3 areas: geometric mean 11.8, 8.5, and 6.7 mg/m^3	Nasal dryness, rhinitis, sinusitis, nosebleed, dermatitis, dizziness, insomnia, reduced leukocytes.	Li, 1996
125 workers, 120 controls, 12.6 yr work history, mean of 3.79 mg/m^3 at time of study	Insomnia, nausea, loss of appetite, dysmenorrhea in female workers, increased peripheral blood lymphocyte chromosomal aberrations in smoking workers vs. control smoking workers.	Xu et al. 1997

Caprolactam Exposure	Response	Reference
Human case reports of occupational exposure (Detailed summaries in Section 5.1)		
1 worker, 3-days to unknown exposure, but caprolactam coated his clothing and skin	Dermatitis of hands and feet, nausea and vomiting, leukocytosis, tonic-clonic seizures.	Tuma et al. 1981
3 workers, unknown exposure, but caprolactam dust covered clothing	Dizziness, nausea and vomiting, tonic-clonic seizures, opisthotonus, brief coma. Caprolactam in urine: 2.9-3.7 g/L	Chen, 2002
2 workers, 2-4 day exposure to unknown concentration	Dermatitis of hands and feet, nausea and vomiting, leukocytosis, hyperglycemia, tonic-clonic seizures.	Woo et al. 1998
Sensitization studies and allergic contact dermatitis reports (Detailed summaries in Section 5.1)		
Patch test of 6 normal participants with 5% caprolactam solution, repeated daily for 4 days	No skin irritant effects produced	Goldblatt et al. 1954
1% aqueous caprolactam solution applied to 3 participants	No skin irritant effects produced	Haskell Laboratory, 1950
Patch test of 204 participants with 3-5% caprolactam solution	No skin irritant effects produced	Summarized by U.S. EPA, 2009
Worker with 29 yr experience at Nylon-6 factory	Presented with dermatitis for last 18 months; patch test with 5% aqueous caprolactam solution positive for allergic contact dermatitis.	Aguirre et al. 1995
Suture thread made of Nylon-6 used in patient that had undergone 40 operations for removing skin tumors	Patch testing with caprolactam solution positive for allergic contact dermatitis.	Hausen, 2003

6.2 Chronic Toxicity to Infants and Children

No toxicity studies were located regarding prolonged animal inhalation exposure to caprolactam beginning at a young age.

In an animal three-generation developmental study, reductions in body weight and food consumption were not found in first-generation (P_1) rats exposed to caprolactam in feed, but were observed in the second- (P_2) and third-generation (P_3) rats treated with caprolactam (Serota et al., 1988). The P_1 rats were young adults (approximately 6 weeks old) upon initiation of treatment. Since the P_2 and

P_3 animals were exposed both *in utero* and through the early growth phase, the decreased body weights noted in the P_2 and P_3 animals were most likely due to the time in the life span at which treatment began.

6.3 Chronic Toxicity to Experimental Animals

Only a few peer-reviewed, multi-day inhalation studies were found in the literature, and no chronic inhalation studies have been performed. Only one comprehensive subchronic inhalation study by Reinhold et al. (1998) has been conducted and is assessed below. Multi-day inhalation and long-term oral studies are also reviewed, many of which were unpublished[1] industry studies, in order to provide a more complete picture of toxic effects resulting from long-term exposure to caprolactam.

Reinhold et al. (1998) subchronic inhalation study

In a 13-week study, Sprague-Dawley CD rats (10/dose/sex) were exposed to caprolactam aerosol (mass median aerodynamic diameter = 3 µm; geometric standard deviation = 1.7) at a concentration of 0, 24, 70, and 243 mg/m^3 for 6 hours/day, 5 days/week (Reinhold et al., 1998). A second group of rats (10/dose/sex) was similarly exposed but euthanized following a 4-week clean air recovery period. Beginning the second week of exposure, treatment-related increases in respiratory (labored breathing) and secretory (nasal discharge) signs were noted in all groups during the caprolactam exposures (Table 7). The quantitative data presented in Table 7 were obtained from the original Huntingdon Life Sciences report by Hoffman (1997) from which the peer-reviewed Reinhold et al. (1998) study was derived. The number of animals exhibiting labored breathing decreased with time in the low- and mid-dose animals, and was not apparent in these two groups after the 36th exposure (approximately week 8 of exposure). Anywhere from 2 to 10 percent of the high exposure animals exhibited labored breathing up to the end of exposure at 13 weeks.

Detailed weekly physical exams noted an exposure-related trend toward an increased incidence of red staining (facial area), clear nasal discharge, and moist rales (Table 8). The incidence of moist rales was highest between the second and eighth week of exposure, where up to 9 out of 40 rats in the 243 mg/m^3 exposure group exhibited this symptom. None of the rats in the 24 mg/m^3 exposure group displayed moist rales during the weekly physical exams.

[1] unpublished means not published in a peer reviewed journal

Table 8. Summary of Significant Findings from In-Life Physical Examinations and Daily Observations, Males and Females Combined[a]

	Exposure Group (mg/m^3)			
	0	**24**	**70**	**243**
In-life physical exam findings at week 13				
# exhibiting condition out of 40 animals				
General animal condition within normal limits	21	14	8	0
Red facial stains	1	10	17	24
Clear nasal discharge	7	11	20	32
Moist rales	0	0	1	3
In-chamber observations, 6th to 26th exposure				
Percentage of animals exhibiting symptoms[b]				
Labored breathing	0	8.1	12.9	17.0

[a] The data in this Table was obtained from the Huntingdon Life Sciences industry report by Hoffman (1997)
[b] Animals exhibiting labored breathing presented as a mean percentage because the number of animals observed daily varied anywhere from 20 to 40 animals.

A neurotoxicity evaluation was conducted just prior to sacrifice based on a functional observational battery including tests for neuromuscular function and coordination, central nervous system activity and excitability, sensorimotor responses, and autonomic function. No evidence of neurotoxicity was observed based on these observational criteria.

At the 13-week terminal sacrifice, no evidence of ophthalmoscopic lesions, clinical pathology, organ weight changes, or macroscopic pathology was observed. Microscopic evaluation by Reinhold et al. observed treatment-related changes only in the nasoturbinal tissues and the larynx and are presented in Table 9. No apparent treatment-related microscopic changes were observed in other regions of the respiratory system including the trachea, main stem bronchi and lungs. Table 9 also shows the type of the nasal and laryngeal tissue lesions, and the pathologist grading of the severity of those lesions. The graded responses in males and females were similar, so the data were combined.

In the nasal region, respiratory epithelium showed a treatment-related increase in goblet cell hypertrophy/hyperplasia, and olfactory epithelium showed a treatment-related increase in incidence of intracytoplasmic eosinophilic material. In almost all of the control animals minimal changes were observed in the respiratory mucosa (19 of 20 rats), and minimal or slight changes were observed in the olfactory mucosa (17 of 20 rats). Thus, the increased severity of the nasal responses with increasing caprolactam concentration was superimposed on the low-level changes that were present in nearly all rat groups. In the larynx, no lesions were apparent in the control animals (Table 9). With caprolactam exposure, laryngeal tissues manifested a dose-related increased incidence and severity of squamous or squamoid metaplasia or hyperplasia of the pseudostratified columnar epithelium covering the ventral seromucous gland. In

five rats exposed to the highest caprolactam concentration of 243 mg/m^3, minimal laryngeal keratinization of the metaplastic epithelium was observed.

Table 9. Summary of Findings in Nasoturbinal and Laryngeal Tissues at the 13-Week Terminal Sacrifice, Males and Females Combined[a]

	Exposure Group (mg/m^3)			
	0	**24**	**70**	**243**
Number Examined	20	20	20	20
Nasal respiratory mucosa[b]				
No change[c]	1	0	0	0
Minimal	5	7	2	0
Slight	14	9	9	8
Moderate	0	4	9	12
Nasal olfactory mucosa[d]				
No change[c]	3	5	2	0
Minimal	17	13	10	3
Slight	0	2	6	3
Moderate	0	0	2	10
Moderately severe	0	0	0	4
Laryngeal tissue[e]				
No change[c]	20	15	8	0
Minimal	0	5	12	12
Slight	0	0	0	8

[a] Nasal and larynx lesions were categorically graded in Reinhold et al. on a scale from lowest to highest severity, as minimal, slight, moderate, or moderately severe. Further quantitative description and statistical analysis of the pathology findings was not presented.
[b] Goblet cell hypertrophy/hyperplasia
[c] Grade labeled “No Change” was not specifically presented in the original pathology tables of Reinhold et al., but was inferred by OEHHA as the number examined minus total number of animals exhibiting minimal or greater cellular changes
[d] Intracytoplasmic eosinophilic material in epithelial cells
[e] Squamous/squamoid, metaplasia/hyperplasia of the pseudostratified columnar epithelium covering the ventral seromucous gland.

Benchmark concentration (BMC) modeling was performed on the treatment-related upper respiratory tract endpoints shown in Table 8 using U.S EPA benchmark dose modeling software (2009c). Only the moderate grade or above lesions in each exposure group were used for BMC modeling of nasal respiratory mucosal lesions because these changes were of a higher severity grade than any of the control lesions (the latter were likely to be age-related). In other words, the moderate and above severity categories of nasal changes were designated as an exposure-related effect for the purposes of the BMC analysis. The resulting dichotomous dose-response input data for BMC modeling for the 0, 24, 70 and

243 mg/m^3 exposure groups were: 0 out of 20 rats, 4 out of 20 rats, 9 out of 20 rats, and 12 out of 20 rats, respectively.

A similar approach was taken in modeling nasal olfactory mucosal changes, where minimal grade age-related lesions were found in the control animals (Table 9). Thus changes greater than minimal (slight, moderate, and moderately severe) were treated as the exposure outcome in BMC modeling. The resulting dichotomous dose-response data for the olfactory mucosa lesions in the 0, 24, 70 and 243 mg/m^3 exposure groups were: 0 out of 20 rats, 2 out of 20 rats, 8 out of 20 rats, and 17 out of 20 rats, respectively.

No age-related lesions were observed in laryngeal tissue of control animals (Table 9). For BMC modeling of laryngeal tissue change, therefore, minimal and slight grades of the non-keratinized lesions were combined for the analysis. The resulting dichotomous dose-response data for the laryngeal tissue lesions in the 0, 24, 70 and 243 mg/m^3 exposure groups were: 0 out of 20 rats, 5 out of 20 rats, 12 out of 20 rats, and 20 out of 20 rats, respectively.

$BMCL_{05}$s (the 95% lower confidence limit of the dose producing a 5% response rate) for the nasal respiratory and olfactory changes and the non-keratinized laryngeal tissue changes found at terminal sacrifice are shown in Table 10. The BMC modeling results used to derive the $BMCL_{05}$s is presented in Appendix A. The $BMCL_{05}$ is used as the point of departure for REL derivation. Using a $BMCL_{05}$ as a point of departure is particularly advantageous when exposure data does not clearly manifest a NOAEL, as is the case with the data from Reinhold et al. (1998) (Table 9). For each endpoint, the $BMCL_{05}$ was derived from the models that provided the best visual and statistical fit to the data, particularly in the low dose region of the line where the $BMCL_{05}$ resides. Following U.S. EPA guidelines, we chose the model with the lowest AIC (Akaike information criterion) in instances where various model fits to the data were similar.

Table 10. $BMCL_{05}$s for the toxic endpoints in the 13-week inhalation exposure study in rats (Reinhold et al., 1998).

Endpoint	$BMCL_{05}$ (model)	BMC_{05}[a] (mg/m^3)	*P* Value	AIC
Nasal respiratory mucosa lesions	3.6 mg/m^3 (log-logistic)	5.9	0.78	77.53
Nasal olfactory mucosa lesions	12 mg/m^3 (log-probit)	17	0.99	60.85
Laryngeal tissue lesions	2.8 mg/m^3 (multistage)	5.3	0.94	53.59

[a] The BMC_{05} represents the modeled concentration resulting in a 5% response rate for the endpoint

Table 11 displays the frequency of upper airway lesions present in the 4-week recovery group. The nasal mucosal changes related to injury due to caprolactam exposure (that is, at a level above that seen in controls) were still apparent in the

two highest exposure groups, indicating incomplete recovery following 4-weeks in clean air. Moderate grade goblet cell hypertrophy/hyperlasia in the nasal respiratory mucosa was still present in rats exposed to 70 and 243 mg/m^3. Moderate and moderately severe intracytoplasmic eosinophilic material was still present in the 70 and 243 mg/m^3 groups. In the laryngeal tissue, recovery of the high exposure group also was not complete at 4-weeks post-exposure. Only one rat in the mid-dose group showed squamous/squamoid metaplasia/hyperplasia of the pseudostratified columnar epithelium. Keratinization of the metaplastic epithelium was absent in the four-week recovery group.

Table 11. Incidences of microscopic findings of nasoturbinal and larynx lesions in the 4-week recovery group[a]

	Exposure Group (mg/m^3)			
	0	**24**	**70**	**243**
Number Examined	20	20	20	20
Nasal respiratory mucosa[b]				
No change[c]	1	2	2	1
Minimal	10	9	4	4
Slight	9	9	8	10
Moderate	0	0	6	5
Nasal olfactory mucosa[d]				
No change[c]	2	1	3	0
Minimal	15	17	10	1
Slight	2	2	3	4
Moderate	0	0	4	13
Moderately severe	0	0	0	2
Laryngeal tissue[e]				
No change[c]	20	20	19	17
Minimal	0	0	1	3

[a] Nasal and larynx lesions were categorically graded in Reinhold et al. on a scale from lowest to highest severity, as minimal, slight, moderate, or moderately severe. Statistical analysis of the pathology findings was not presented.
[b] Goblet cell hypertrophy/hyperplasia
[c] Grade labeled “No Change” was not presented in the original pathology tables of Reinhold et al., but was inferred by OEHHA as the number examined minus total number of animals exhibiting minimal or greater micropathological changes
[d] Intracytoplasmic eosinophilic material in epithelial cells
[e] Squamous/squamoid, metaplasia/hyperplasia of the pseudostratified columnar epithelium covering the ventral seromucous gland.

Reinhold et al. (1998) report that the background incidence of the nasoturbinal findings were considered by the pathologist to be within normal limits for test animals of this age and strain. The increase in incidence and severity of the nasoturbinal and squamous metaplastic/hyperplastic laryngeal findings in

caprolactam-treated rats was stated to be a “localized adaptive response to a minimal irritant effect” and attributed to particulate exposure rather than an adverse toxicological response to the test material in the nasal passages. The only toxicologically relevant finding considered by the authors due to caprolactam exposure was the keratinization in the larynx; they viewed 70 and 243 mg/m^3 as a NOAEL and a LOAEL, respectively.

Because the authors did not consider the nasal and laryngeal changes relevant for toxicological consideration, further review of these particular types of lesions by other pathologists is summarized below.

Increased goblet cell (i.e., mucous cell) hypertrophy/hyperplasia in respiratory mucosa has been frequently observed in the anterior nasal cavity of rodents in response to repeated inhalation of irritants (Renne et al., 2007; Renne et al., 2009). It develops from hypertrophic epithelium with typical goblet cells distended with secretory droplets. This region of the nose is one of the most sensitive sites in rodents due to high volume of air flow through the ventral aspect of the nasal cavity over the nasal and maxillary turbinates.

Eosinophilic deposits, or globules, like those found in the olfactory epithelial cells of the Reinhold et al. study occasionally have been described by other researchers in otherwise normal epithelium of untreated rats, but such changes are more frequently observed in aged animals (Morgan, 1991; Harkema et al., 2006; Renne et al., 2009). These deposits occur often in the epithelial sustentacular cells and are considered dilated endoplasmic reticulum containing proteinaceous material and not a dysplastic (i.e., abnormal) alteration. They have often been referred to as hyaline droplets or hyaline degeneration (Harkema et al., 2006). The lesion increases in severity and extent with age and exposure to specific irritants, such as dimethylamine and cigarette smoke (Morgan, 1991). The mechanism by which such lesions appear in aging rats, and the nature of the response to irritants, is not understood. Intracellular eosinophilic deposits also have been observed in other studies in nasal respiratory mucosa and in other respiratory tract epithelium (Morgan, 1991; Renne et al., 2009), but either was not found in the Reinhold et al. animals, or was found in comparable incidence and severity in rats from both control and exposure groups.

The region of the larynx investigated by Reinhold et al.(1998), the pseudostratified columnar epithelium on the ventral floor of the larynx at the base of the epiglottis, is especially sensitive to inhaled materials (Renne and Gideon, 2006; Renne et al., 2009). Squamous metaplasia as noted by Renne et al. (2009) may occur in association with acute and/or chronic inflammation or in the process of regeneration. Laryngeal squamous metaplasia has been characterized as a classic example of indirect metaplasia (Osimitz et al., 2007). Inhalation of an irritant damages sensitive respiratory or transitional epithelium, so that cells that proliferate to replace the lost cells produce a replacement epithelium that is better adapted to the new environment.

In an expert workshop to evaluate larynx squamous metaplasia, a similar conclusion was made. This type of epithelial change is a result of transformation of the pre-existing epithelium to a squamous epithelium, with or without keritinization (Kaufmann et al., 2009). The lesion was classified as the morphologic correlate of an adaptive process from a more sensitive to a more resistant type of epithelium, which is indicative of local irritation. Focal, minor metaplastic changes that may also occasionally occur in control animals were considered "non-adverse", while moderate to severe squamous metaplasia should be considered adverse as it may be associated with dysfunction. In humans, this dysfunction may result in hoarseness and an altered coughing reflex. In the rats exposed to caprolactam, exposure to the low and mid-level concentrations resulted in only a "minimal" grading for larynx metaplasia (Table 9).

For an assessment of adversity (equivalent term to "toxicity"), Kaufman et al. (2009) felt it was more relevant to observe dysfunction of an organ or tissue (e.g., by test designed to measure mucociliary clearance). For the rats in the Reinhold et al. (1998) study, adverse effects were apparent in terms of treatment-related increases in labored breathing, nasal discharge, red staining of the facial area, clear nasal discharge, and moist rales that began after approximately 1-2 weeks of exposure.

An earlier paper by Osimitz et al. (2007) suggested that laryngeal squamous metaplasia should not be used as an endpoint for quantitative risk assessment, as it is well-differentiated, reversible, and generally lacking signs of progression. This, in the opinion of the workshop panel cited earlier (Kaufmann et al., 2009), was not an approach supported by data. All available information, according to that expert panel, should be carefully considered by the pathologist, including other related health effects that are evaluated as "adverse".

Other multi-day inhalation exposure studies

Several published and unpublished[1] multi-day inhalation studies have been conducted with caprolactam. However, these studies generally lacked complete methodology descriptions and only provided brief overviews of their findings. As much pertinent data are summarized below as could be found for each of these studies.

Goldblatt et al., (1954) exposed three guinea pigs to 118 - 261 mg/m^3 caprolactam "dust" for 7 hr/day for 7 days and reported no adverse effects other than occasional cough. No other toxicological exams were apparently performed, other than observing for signs of irritation. The majority of the caprolactam particles formed for the study were reported to be below 5 µm in size.

[1] unpublished means not published in a peer reviewed journal

Hohensee et al. (1951) exposed up to 10 guinea pigs to 51 mg/m^3 caprolactam 5-8 hr/day for 26-30 days. No external pathologic changes or evidence of convulsions were noted during the exposures. Pathological and histological examination of a few of the animals revealed compound-related slight inflammation of the nasal mucosa and tracheal mucosa. However, no information was provided on the nature or extent of the inflammation or whether controls were free of this involvement.

In a multi-day unpublished industry study, two rats were exposed to a nominal concentration reported as 3000 ppm (equivalent to 13,900 mg/m^3 caprolactam particles for 2 hrs, followed five days later by a series of five nominal 1 to 2 hr exposures ranging from 2700 to 6800 ppm (equivalent to12,500 to 31,500 mg/m^3 on successive days (Haskell Laboratory, 1950). The exposure concentrations were expressed in ppm in the report. OEHHA notes that particle exposures should be expressed in mg/m^3 as shown above in parentheses. Nominal exposure entails calculating the loss of material to the gassing chamber when heated and the rate of air flow. No direct measurement of airborne caprolactam concentration is performed. Nominal estimates of air concentration often over-estimate actual air concentrations. General discomfort and inflammation around the eyes and nose were observed during the exposures. Gross and microscopic pathological examination three days following the last exposure showed slight lung edema and congestion of the spleen, but no pathology in any other organs.

In another unpublished[1] study, 4 dogs, 6 guinea pigs, 6 rats and 2 rabbits were exposed subchronically to caprolactam fumes generated by heating the chemical in air. This study was conducted in 1952-53 and only recently reported to the U.S. EPA (2009a). The composition of the fume was not evaluated and it was unclear from the report if the exposures were nominal or dynamic exposures. The authors and laboratory conducting the experiment are not identified and the document was labeled ‘company sanitized’. All animals were exposed to 444 mg/m^3 6 hrs/day for 43 exposures. Half of the guinea pigs, rat and rabbits, and 3 of the dogs, were then exposed to 1020 mg/m^3 on exposures 44 through 67 or 73.

In dogs, the fumes seemed to aggravate open sores and especially infections and soreness of the eyes (U. S. EPA, 2009a). Two of the four dogs displayed occasional muscle tremors during exposure to the low concentration of caprolactam. One of these dogs displayed severe muscle tremors, weakness, coughing with a dense white froth around the mouth when exposed to the high concentration during exposures 46 through 67. In all cases, the dogs were normal the next morning after exposure. One dog had a significant lowering of systolic pressure and pulse pressure but otherwise no other significant changes in weight, blood sugar, cholesterol, BUN, thymol turbidity or hematology. Gross pathology showed an indication of either acute duodenitis or gastroenteritis in two dogs, but it was suggested this was an aggravation of an existing gastro-intestinal

[1] unpublished means not published in a peer reviewed journal

disorder. Microscopic examination revealed no changes that were attributable to caprolactam.

The study reported that one rabbit of the two rabbits exposed showed slight corneal damage and both rabbits showed mild irritation of the conjunctiva in both eyes (U. S. EPA, 2009a). No gross or microscopic pathology was observed in the rats or rabbits. In guinea pigs, one of the six had a lung reaction to a foreign body and a kidney showed evidence of regeneration of tubules. Another guinea pig had nephritis. A third guinea pig displayed consolidation of the apex of the right lung. No other gross or microscopic changes were detected in the remaining 3 guinea pigs.

Long-term oral exposure studies

Although no chronic inhalation experimental animal exposure studies have been conducted for caprolactam, the NTP (1982) performed a chronic oral exposure study in rodents examining both cancer and noncancer endpoints. A comprehensive 90-day oral exposure study in dogs is also summarized to provide toxicological information for a non-rodent species.

A two-year caprolactam carcinogenesis bioassay feeding study was conducted by the NTP (1982). Caprolactam was incorporated in the diet of male and female rats at concentrations of 3,750 ppm or 7,500 ppm, and in the diet of male and female mice at concentrations of 7,500 and 15,000 ppm. Mean body weights of all dosed groups were decreased compared to their respective control groups throughout the two-year study. Feed consumption was inversely related to dose in rats, but caprolactam in the feed of mice had no effect on feed consumption. Growth curves for rats and mice are presented in graphical form by the NTP, but statistical analysis on mean body weight gain and feed consumption was not performed. The NTP concluded that the dose-related decrements in mean body weight gains indicate that it is highly likely that animals in the study were receiving the maximum tolerated doses of caprolactam.

Histopathologic examination did not find any compound-related effects in nasal tissues, larynx, esophagus, stomach, or any other tissues and organs. Other than the dose-related decrements in feed consumption and body weight gains, the NTP concluded there was no evidence of nonneoplastic lesions associated with oral administration of caprolactam as demonstrated by histopathologic examination of rats and mice in this study. Table 12 presents the estimated range of daily caprolactam intake in feed, assuming 100% absorption, for each dose group during the study.

Table 12. Estimated range of daily intake[a] of caprolactam in mg/kg body weight during a two-year feeding study (NTP, 1982).

Species	Males		Females	
	Low Dose	High Dose	Low Dose	High Dose
Rat	210 - 400	400 - 670	260 - 370	440 - 670
Mouse	790 - 1100	2200 - 2400	1200 - 1800	3100 - 3900

[a] Caprolactam intake range for each dose group of each species was based on a week in the second year of the study with the lowest mg/kg body weight intake, and on week 4 feed consumption, the period of growth with the highest mg/kg body weight intake.

An unpublished[1] 90-day oral exposure study in beagle dogs conducted by Burdock et al. (1984) resulted in analogous findings to those observed in rats and mice of the NTP study. Three groups of eight dogs (4 each per sex) were fed at dose levels of 0.1%, 0.5% and 1.0% caprolactam in the diet. These percentages of caprolactam in the diet were equivalent to an average of 32, 164 and 292 mg/kg/day caprolactam consumed by males, respectively, and 33, 158, 389 mg/kg/day caprolactam consumed by females, respectively. A control group of equal size was fed a basal diet only. At the conclusion of 90 days on study, the only significant finding was a slightly lower mean body weight of the 1.0% females compared to controls. A similar change was not observed for the males, and total food consumption was comparable between groups of both sexes. No difference from controls was observed in absolute or relative organ weights for any group. Gross and microscopic examination of tissues and organs revealed no remarkable findings. Ophthalmologic findings were negative. No dose-related changes were observed for hematologic and serum chemistry samples.

7. Developmental and Reproductive Toxicity

Gross (1984) summarized the foreign language literature, almost exclusively in Russian, examining the gynecological effects of caprolactam in female workers. Most of this work was published between the 1950s and 1970s. Specific caprolactam exposure concentrations were not given, although in one instance concentrations between 100 to 400 mg/m^3 were reported during the charging and pouring of the melting tanks, and between 1 to 10 mg/m^3 at other times. The abnormalities found in excess over those of the control groups consisted of dysmenorrhea (i.e., painful menstruation) and menorrhagia (i.e., excessive uterine bleeding), oligomenorrhea (i.e., markedly reduced menstrual flow), and prolongation of the exfoliative phase. The obstetrical complications that were found to be excessive compared to those of controls consisted of post-partum hemorrhage, toxemia of pregnancy, premature birth, and inadequate uterine contractions during labor.

Gross (1984) noted that high temperatures, high humidity and noise level were likely contributory factors to the abnormalities described in female workers. Co-exposure to other chemicals, including dinyl oxides, benzene, cyclohexane,

[1] unpublished means not published in a peer reviewed journal

cyclohexanol, cyclohexanone, acetone, and trichloroethylene, were also possible contributory factors.

In a more recent investigation, a retrospective reproductive and development study was conducted in 312 female workers in a Chinese Nylon-6 manufacturing facility (Liu et al., 1988). This study was translated from Chinese into English by OEHHA. The workers were compared to a group of 302 female workers in a textile factory with no contact with caprolactam or other chemicals. The noise level in the two facilities was similar. All workers had worked in the facility for more than one year, and caprolactam exposure was said to be below 10 mg/m^3, except for a few measurements slightly higher than this level. Specific exposure concentrations were not provided. In the caprolactam-exposed women, primary infertility ($0.005<p<0.05$), and pregnancy hypertension ($p<0.005$) was greater compared to the control group. Other measures of pregnancy function and fetal development were similar to controls. Abnormal menstrual function, including abnormal menstrual cycles, was higher in the caprolactam-exposed female workers ($p<0.005$), although no difference was seen between the two groups regarding symptoms of dysmenorrhea.

No studies were located investigating the developmental and reproductive toxicity of caprolactam by the inhalation route in experimental animals.

In oral exposure studies, (Gad et al., 1987) dosed pregnant rats by gavage with caprolactam at 100, 500, or 1,000 mg/kg/day on gestation days 6-15. Increased maternal mortality was observed at the highest dose. A dose-related decrease in mean maternal body weight was observed with a statistically significant reduction ($p\leq0.05$) in total body weight at the highest dose level (a 10 and 11% reduction on gestational days 15 and 20, respectively). A statistically significant reduction ($p\leq0.05$) in mean weight change was observed during the treatment period at the two highest doses (5.2 and 2.3% mean weight gain at the mid- and high-dose, respectively, compared to a 13.4% weight gain for the control group). Food consumption was reduced in the two highest dose groups. The mean incidence of resorptions was increased at the highest dose.

No dose-related skeletal anomalies or major malformations were noted among the offspring of any exposure group. An apparent dose-related increase in the mean number of skeletal variants per litter was observed, including incomplete ossification of the skull or vertebral column and the presence of extra ribs. However, no statistically significant difference in skeletal variation values between treated groups and the control group were noted.

Gad et al. (1987) also dosed pregnant rabbits by gavage with caprolactam at 50, 150, or 250 mg/kg/day on gestational days 6-28. Sixteen percent mortality and statistically significantly decreased overall maternal body weight gain were observed at the highest dose. Corrected weight gain (i.e., weight gain minus weight of gravid uterus) was statistically significantly lower ($p<0.05$) from day 6 to

29 of gestation in the 150 mg/kg group. Absolute maternal body weights were unaffected in this mid-dose group. Mean fetal weights were statistically significantly reduced ($p<0.05$) by 12% in each of the two highest dose groups compared to controls. The incidence of major malformations was unaffected by caprolactam treatment. Minor skeletal anomalies included an increased incidence of unilateral or bilateral thirteenth ribs in the highest dose group.

Gad et al. (1987) concluded that caprolactam given by gavage to two species up to levels that caused severe maternal toxicity did not support a finding of the compound causing either embyotoxicity or teratogenicity. Fetotoxicity was evidenced in rabbits by lower fetal weights at the two highest doses, and an increased incidence of 13th ribs at the highest dose level.

In a multi-generation study, rats were given a 1,000, 5,000, or 10,000 mg caprolactam/kg diet (ppm) over three generations (Serota et al., 1988). Each generation was treated over a 10-week period. Consistently lower mean body weights and food consumption were observed in both P_2 and P_3 parental generations at 5,000 and 10,000 ppm, but body weights were unaffected in P_1 animals at all dose levels. The mean body weight changes were statistically significant ($p\leq0.05$) in all high dose groups at all time points with weight reductions in both males and females ranging from 10 to 21% compared to controls. For mid-dose animals, a statistically significant change in mean body weight occurred only in P_2 males, a 13% reduction compared to controls, during week four of exposure.

Dose-related reductions of fetal body weights were observed in all filial generations. For example, statistically significant differences ($p\leq0.05$) noted in F_{1a} and F_{1b} high dose groups (17 to 23% reductions compared to controls) and occasionally in mid-dose groups (11 to 14% reductions in F_{1b} offspring only compared to controls). Based on mean body weight and mean food consumption values at week 10 in P_1 females, caprolactam in the diet at 1000, 5000 and 10,000 ppm was equivalent to a daily dose of 700, 3500 and 5600 mg/kg caprolactam, respectively.

No treatment-related effect on gross appearance, gross pathology, survival rate or number of pups was observed. A slight increase in the severity of spontaneous nephropathy was observed on histopathologic examination of males in the high-dose group of the first parental generation.

Serota et al. (1988) concluded that caprolactam in the diet at the two highest exposures resulted in decreases in body weight in both pups and parental animals in utero through weaning. Similar effects on food consumption were also noted. Body weights were unaffected in P_1 animals at all dose levels, and reduced food consumption was observed only at week 10 in P_1 females. No effects were evident on reproductive performance or offspring survival, and only minimal kidney toxicity was observed in males at the highest dose level.

In other oral exposure studies, Salamone (1989) reported no sperm abnormality in male mice treated with 222, 333, 500, 750, or 1,125 mg/kg caprolactam by gavage daily for five days, although mortality was evident at the highest dose. Following the fifth gavage with 1,125 mg/kg caprolactam, the mice immediately became motionless. In four of the nine mice treated, this inactivity was followed 10 min later by racing around the cage and death within seconds. These deaths are probably related to the method of oral treatment because exposure of mice up to 2200 to 2400 mg/kg caprolactam in feed for two years by the NTP (1982) did not result in an increase in mortality. A similar study in male rats did not observe DNA damage to spermatocytes following an oral dose of 750 mg/kg caprolactam (Working, 1989).

The primary finding of the two developmental/reproductive toxicity oral exposure studies was that caprolactam may be fetotoxic due to reduced fetal body weight. Reductions in fetal weight in the gavage study occurred at the same dose levels that reductions in maternal food consumption and body weight occurred. Based on this gavage study, the concomitant reduction in both maternal body weight and fetal weight make it difficult for OEHHA to conclude that caprolactam is exclusively fetotoxic. However, body weights of P_1 rats in the multi-generation study were not reduced by caprolactam exposure yet resulted in reduced fetal weights in F_{1a} and F_{1b} offspring. This finding indicates a fetotoxic LOAEL of 5000 ppm caprolactam in feed, which is equivalent to a maternal daily dose of 3500 mg/kg. The calculated NOAEL is 700 mg/kg.

Assuming 100% pulmonary absorption, the NOAEL is equivalent to an air concentration of 2500 mg/m^3 (700 mg/kg x 70 kg body wt. / 20 m^3/day) in a route-to-route extrapolation. For comparison, brief human exposures to lower caprolactam concentrations in the range of 1900 to 5600 mg/m^3 (400 to 1200 ppm) have been characterized as extremely irritating, and subchronic exposures of rats to air concentrations as low as 24 mg/m^3 have resulted in labored breathing and nasal secretory discharge. Applying a 100-fold uncertainty factor (10-fold UF each for interspecies and intraspecies extrapolation) for extrapolation from an animal developmental study to human exposure would produce a proposed REL of 25 mg/m^3. The acute and chronic RELs of 50 μg/m^3 and 2.2 μg/m^3, respectively, are considerably lower than that derived from the oral multi-generation animal study.

These findings show that the oral dose at which fetotoxicity occurs is likely not relevant to air concentrations of caprolactam for REL derivation due to upper respiratory tract injury occurring at lower concentrations. The acute, 8-hour and chronic RELs developed in this document based on caprolactam air exposures would be protective for reproductive/developmental effects. Therefore, OEHHA is using pulmonary and sensory irritation endpoints for the caprolactam inhalation RELs.

8. REL Derivations

8.1 Derivation of the Acute Inhalation Reference Exposure Level (1-hour exposure)

As noted above, only two human studies exist that examined the acute sensory irritant effects in association with quantified concentrations of caprolactam. Because of limitations in the occupational study (Ferguson and Wheeler, 1973), an acute REL cannot be derived reliably from this study. The second acute exposure report was the chamber study by Ziegler et al. (2008). OEHHA applied a non-parametric test, Page's trend test, to the individual human data provided to us by Dr. Ziegler, as noted previously. We observed a statistically significant ($p<0.05$) dose-response relationship for eye blink frequency and subjective eye irritation at one hour of exposure. Both measures of sensory eye irritation were increased in subjects exposed to 5 mg/m^3 compared to the non-exposed group.

Walker et al. (2001) suggested that the increased rating of eye irritation and eye blink frequency with exposure to an irritant are manifestations of the same underlying event of ocular trigeminal nerve activation. Objective measures such as eye blink frequency are less susceptible to cognitive biases than subjective ratings of eye irritation. Eye blink frequency also had a more robust response at 5 mg/m^3 than eye irritation, indicating eye blink frequency is a more sensitive measure of caprolactam exposure. Thus, the acute REL is based on increased eye blink frequency, with eye irritation as supporting evidence for the REL.

Study	Ziegler et al., 2008
Study population	20 human adults: 10 male, 10 female
Exposure method	Whole body chamber
Exposure duration	1 hour
Critical effects	Increased eye blink frequency
LOAEL	5 mg/m^3
NOAEL	0.5 mg/m^3
Time adjusted exposure	0.5 mg/m^3 (irritant: no adjustment applied)
LOAEL uncertainty factor	1
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (site of contact; no systemic effects)
Toxicodynamic (UF_{H-d})	10
Cumulative uncertainty factor	10
Acute reference exposure level	0.05 mg/m^3 (0.011 ppm)

We applied a NOAEL/LOAEL approach to the statistically significant increase in eye blink frequency, rather than using benchmark modeling. BMC analysis using continuous model methodology could not fit the eye blink data to a model. Likely,

the low- and mid-exposure levels, with their slight increase in response over control values and large variances, were not different enough from the controls to provide a good curve fit with the available models. We did not apply a time adjustment to the NOAEL from Ziegler et al. since we used the exposure data collected at 1 hour of exposure.

Chemicals that have effects limited to the extrathoracic region (i.e., nose and larynx), including caprolactam, are not predicted to be much different kinetically in children compared to adults when dosimetric adjustments are made (OEHHA, 2008). Thus, no UF_{H-k} is applied for intraspecies toxicokinetic variation among individuals. Only normal individuals without allergic rhinitis or other respiratory symptoms were investigated by Ziegler et al. Thus, a UF_{H-d} of 10 is applied to the REL derivation to address the human variation in the intraspecies toxicodynamic response to respiratory irritants, including potential exacerbation of asthma in children and adults. The total UF = 10 applied to the NOAEL results in an acute REL = 0.05 mg/m^3.

Consistent findings of seizures in heavily exposed adult workers and in experimental animal studies merit concern for exposure in children, who may be more sensitive than adults to chemicals that have neurological effects. OEHHA believes an acute REL protective of eye irritant effects will be sufficient to protect children from the neurological effects, and that an additional UF beyond the cumulative intraspecies UF = 10 is not necessary. Worker exposure to unspecified high levels of caprolactam may produce tonic-clonic seizures, but the exposure levels necessary to cause this neurological effect are above estimated exposures in the range of 22 to 168 mg/m^3 that have resulted in dermal, eye and respiratory irritation in workers (Kelman, 1986; Ferguson and Wheeler, 1973; Hohensee, 1951). In rats, airborne exposure at g/m^3 levels resulted in tremors, while intraperitoneal injections of 900 mg/kg and above produced convulsions. The quantified exposure levels in these animal studies where these neurological effects were found were substantially higher than the NOAEL for eye irritation of 0.05 mg/m^3 derived from the work by Ziegler et al. (2008). The considerably lower NOAEL for eye irritation supports the application of a 10-fold intraspecies toxicodynamic UF as sufficient to protect children from any neurological effects resulting from acute caprolactam exposure.

Subjective eye irritation increased with increasing caprolactam exposure, although the irritation score did not rise sharply with exposure concentration (i.e., mean eye irritation scores were between 0 (not at all) and 1 (barely) for all caprolactam exposures). Typically, when the sensory irritant threshold is reached the graded response should rise steeply. Over a range of hardly more than one order of magnitude of concentration, sensory irritation may increase from “barely detectable” to “painful irritation” (Cain et al., 2006; Nielsen et al., 2007). Such a steep rise in the graded response was not apparent for caprolactam in this study, suggesting the sensory irritant threshold was not reached in all or most

participants of the study, or that eye irritation is not as sensitive a measure of caprolactam exposure as eye blink frequency.

We also observed differences between the control group and the 5 mg/m^3 exposure group for total subjective symptom and complaint score (both with and without the odor subscore). We prefer to base a REL on an objective endpoint (i.e., eye blink frequency) because the total symptom and complaint score are subjective measures and lacked independence for many of the questions. In other words, many of the questions discussed in Section 5.1 above were asking the same question in different ways.

8.2 Derivation of the 8-Hour Inhalation Reference Exposure Level

Study	Reinhold et al. 1998
Study population	Sprague-Dawley CD rats (10 animals/sex/group)
Exposure method	Discontinuous whole-body inhalation exposure to 0, 24, 70, and 243 mg/m^3 caprolactam aerosol
Critical effects	Upper airway lesions of nasal and laryngeal epithelium
LOAEL	24 mg/m^3
NOAEL	Not observed
$BMCL_{05}$	3 mg/m^3
Exposure continuity	6 hours per day, 5 days/week
Exposure duration	13 weeks
Average experimental exposure	1.607 mg/m^3 (3 mg/m^3 x 6/8 x 5/7)
Human equivalent concentration	0.402 mg/m^3 (for extrathoracic respiratory effects, RGDR = 0.25
LOAEL uncertainty factor	1 ($BMCL_{05}$ used as point of departure)
Subchronic uncertainty factor	2 (for 13 wk exposure in rodents) (see below)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1
Toxicodynamic (UF_{A-d})	√10 (default: no toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (site of contact; no systemic effects)
Toxicodynamic (UF_{H-d})	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	60
8-Hour reference exposure level	7 µg/m^3 (1.4 ppb)

The comprehensive subchronic exposure study in rats by Reinhold et al. (1998) is the basis of the 8-hour REL, resulting in a level of 7 µg/m^3 (rounded up to one significant figure from 6.70 µg/m^3), or 1.4 ppb (rounded to 2 significant figures to avoid large rounding errors because the first digit is a 1). The occupational studies of long-term exposure to caprolactam were considered by OEHHA to be inadequate for use as a point of departure for the 8-hour REL, which pertains to repeated 8 hr exposures, and the chronic REL (see Section 6.1 for reviews of the occupational studies).

In deriving 8-hr and chronic RELs, issues concerning the presence of unaccounted gas phase caprolactam in the Reinhold et al. study, and the phase of caprolactam that exists in the ambient air are considered. One of the uncertainties in the Reinhold et al. (1998) study is that the method used to measure the exposure levels in the chamber only captured the aerosol fraction, leaving any of the caprolactam that may have partitioned into the vapor phase unmeasured. This could lead to an underestimation of the respirable caprolactam

in the chamber. If the caprolactam concentration to which the rats were exposed was underestimated, it would mean that the point of departure for the chronic and 8-hour RELs could be too low. Therefore, OEHHA attempted to evaluate how much unmeasured vapor could have been present in the exposure chambers.

In the original Huntingdon Life Sciences report by Hoffman (1997) from which the peer-reviewed Reinhold et al. (1998) study was derived, it was stated that a very minor amount (<3 ppm) of unmeasured caprolactam vapor may have been present in the caprolactam aerosol atmospheres. The analysis of caprolactam by Reinhold et al. used gravimetric sampling to estimate the caprolactam concentrations in the test chambers. Air drawn from the chambers passed through glass-fiber filters mounted open-faced in a filter holder. The gravimetric concentration was calculated based on the weight of the filter papers before and after sample collection, and the known volume of air that passed through the filter papers. Any caprolactam vapor would have passed through the filter papers and not been measured.

There is supporting data that indicates the method used by Reinhold et al. to generate the caprolactam atmospheres produces predominantly caprolactam aerosol and dry particles, and that any additional amount of caprolactam vapor generation is small enough to be disregarded for REL derivation. Nau et al. (1984) investigated the effects of temperature and humidity on sample collection of airborne caprolactam aerosol and fume (a dry suspension resulting from condensation products). In their study, caprolactam was dissolved in water in a 1:1 to 1:0.2 solution (water to caprolactam) by weight and then aerosolized in a test chamber under different conditions of temperature and humidity. The chamber concentrations generated were between 2.7 and 40 mg/m^3. Reinhold et al. (1998) had used a similar method to generate caprolactam aerosols.

Caprolactam sample collection by Nau et al. (1984) consisted of the tandem utilization of a glass fiber filter followed by a XAD-2 resin tube to collect the particles. At the end of the sample train, two water impingers collected any caprolactam that escaped the filter and XAD-2 resin. Presumably, caprolactam gas and some very small caprolactam particles would pass through the glass fiber filter and would be captured in the XAD-2 resin tube or water impingers. Under exposure conditions similar to that used by Reinhold et al. (20-27°C, 21-74% relative humidity), only a mean total of 0.8% of the caprolactam was trapped by the XAD-2 resin tube and water impingers, with about 99% of the caprolactam trapped on the filter and filter support. These data show that estimating the 8-hour and chronic RELs based on the gravimetric concentrations estimated by Reinhold et al. are valid.

The other consideration to be addressed is the proportion of caprolactam found in the gas phase and the diameter range of caprolactam particles that would be found in the ambient air environment following release of caprolactam from an emission source. This information is important in determining the region of the

respiratory tract that would be impacted by inhaled caprolactam. Little evidence could be found in the literature regarding the phase of caprolactam found in the environment, or the size of the particles if they were in the solid phase. In an air quality study by Cheng et al. (2006) caprolactam aerosol was detected on $PM_{2.5}$ filters, but the level of caprolactam gas present in the air, or caprolactam particles greater than $PM_{2.5}$, was not measured.

In the Reinhold et al. study, the authors aerosolized an aqueous caprolactam solution for the exposures, which had an MMAD of 3.0 µm and a geometric standard deviation of 1.7 µm. The mammalian nose is an effective filter for a large fraction of particles above 1 µm in diameter (Stuart, 1984; Swift and Strong, 1996; Yeh et al., 1996). So it is not surprising that upper respiratory system was the target of caprolactam exposure.

At the level of the 8-hour and chronic RELs, it can be expected that a significant fraction of caprolactam would be in a gas phase because the RELs are considerably below the airborne saturation level of 13 mg/m^3. Very water-soluble gases, including caprolactam, also deposit in the upper respiratory tract (OEHHA, 2008). However, if very small caprolactam particles are released or formed in the ambient air, some may bypass the upper respiratory system when inhaled and deposit largely in the tracheobronchial regions, or even reach the alveoli. The available data in experimental animals indicate the upper respiratory system is the target of inhaled caprolactam, unless exposure to massive amounts of caprolactam occurs. No occupational studies have documented pulmonary dysfunction (i.e., lower respiratory tract) as a result of caprolactam exposure. Based on the limited data available, OEHHA concludes that both vapor and particle phase caprolactam predominantly deposits in the upper respiratory tract, and that using the most sensitive endpoint of upper respiratory tract lesions determined by Reinhold et al. (1998) should be considered protective of the lower respiratory tract as well.

The 8-hr REL derivation is based on a $BMCL_{05}$ = 3 mg/m^3 (rounded from 2.8 mg/m^3, see Table 9) for a pathology grading of minimal and slight increases in squamous/squamoid metaplasia/hyperplasia in the larynx of male and female rats exposed to caprolactam aerosol for 13 weeks (Reinhold et al., 1998). The $BMCL_{05}$ for exacerbation of changes to the respiratory and olfactory nasal mucosa resulted in essentially the same value (respiratory $BMCL_{05}$ = 4 mg/m^3) or was slightly greater (olfactory $BMCL_{05}$ = 12 mg/m^3).

Reinhold et al. (1998) regarded laryngeal keratinization of the metaplastic epithelium to be the primary adverse effect, resulting in a NOAEL of 70 mg/m^3. The other effects in the upper respiratory system were considered by the researchers to be normal adaptive responses to an irritant, which they did not consider a toxicological endpoint. However, OEHHA RELs include health protection against mild irritant/inflammatory effects. These types of mild inflammatory changes are primary endpoints of toxicity as indicated in the

Revised Air Toxics Hot Spots Program Technical Support Document for the Derivation of Noncancer Reference Exposure Levels and RELs for Six Chemicals (OEHHA, 2008). The irritant-related microscopic changes in the upper respiratory tract, combined with the observations of respiratory irritation/inflammation (nasal discharge, moist rales, red staining around the face) and labored breathing in all caprolactam-treated groups presented in detail in the original Huntingdon Life Sciences report by Hoffman (1997), support the lack of an observed NOAEL in the principal study.

The $BMCL_{05}$ = 3 mg/m^3 was adjusted to an average experimental exposure of 1.6 mg/m^3 for eight-hour exposures, seven days/week. The concentration at the $BMCL_{05}$ is below the saturated vapor concentration of caprolactam of 13 mg/m^3 at 25ºC. Thus, a greater proportion of caprolactam may be in gaseous form rather than the aerosol form used in the study. Although no studies have been conducted comparing the potency of gaseous or aerosol forms of caprolactam, the evidence in this document indicates both forms are expected to have the same toxicological endpoints.

Given that the predominant form humans will be exposed to at the level of the RELs will likely be the gaseous form, a regional gas dose ratio (RGDR) approach will be used for the human equivalent concentration (HEC) adjustment. The RGDR of 0.25 was calculated using US EPA methodology (OEHHA, 2008) for extrapolation from rat and human exposure. The equation for gases with respiratory effects is:

$$RGDR = (MVa/MVh) / (SAa/SAh) \qquad \text{Eq. 8-1}$$

Where:

MVa = animal minute volume
MVh = human minute volume
SAa = animal surface area for lung region of concern
SAh = human surface area for lung region of concern

Surface areas for the region of concern, the extrathoracic region, for rat (15 cm^2) and human (200 cm^2) were obtained from Table F.1.1 in OEHHA (2008). Minute volume for rats was calculated using Eq. 8-2 below, using intercept (b_0) and slope (b_1) values from Table F.1.2 in OEHHA (2008). Body weight (BW) for both male and female rats combined (0.323 kg) was averaged over the 13-week exposure duration from body weight tables in Reinhold et al. (1998) and the authors' unpublished[1] industrial data (Hoffman, 1997).

$$\log_e(MV) = b_0 + b_1 \log_e(BW) \qquad \text{Eq. 8-2}$$

Where

BW = 0.323 kg; b_0 = -0.578; b_1 = 0.821

[1] unpublished means not published in a peer reviewed journal

$\log_e(MV) = -0.578 + 0.821 \log_e(0.323\ kg)$
MV = 0.222 L/min or 222 ml/min

For humans, an average adult male and female combined MV of 11,944 ml/min was estimated using breathing rate data from US EPA (2009b).

Thus:

RGDR = (222 ml/min/11,944 ml/min) / (15cm^2/200 cm^2)
RGDR = 0.0186 / 0.075
RGDR = 0.25

A subchronic UF = 2 was incorporated into the REL derivation for extrapolation from 13-week exposure in the rats to chronic exposure. Although 13 weeks of exposure is 12.5% of the 2-year life expectancy of rats, which would entail use of a subchronic UF = 1 for >12% of lifetime exposure, U.S. EPA (1994a) recommends using a subchronic adjustment factor for all 13-week studies regardless of species. OEHHA has typically used a subchronic UF = √10 for 13-week exposure studies in rats and mice. However, for rodent studies of this exposure duration a subchronic UF = 2 for upper respiratory irritants, when the resulting injury is considered mild, is appropriate.

The basis for using a subchronic UF = 2 was derived from the numerous rodent studies with formaldehyde (OEHHA, 2008). Comparison of 13-week exposure studies with studies of longer duration up to 2 years shows that the NOAELs and LOAELs for upper airway injury are often the same, with only a 2-fold difference between chronic and 13-week study NOAELs and LOAELs in some cases. The 2-fold lower NOAELs and LOAELs were often a result of the choice of the formaldehyde exposure concentration used in the studies.

The severity of the upper respiratory tract injury also supports a subchronic UF = 2. The pathology grading of the upper respiratory tract resulting from caprolactam exposure indicates only a mild increase in injury. The exacerbation by caprolactam exposure of normal nasal olfactory tissue degeneration was small, increasing from minimal to slight at the lowest dose of 24 mg/m^3. The laryngeal tissue damage caused by caprolactam was minimal, at best, at the low dose. Overall, only a few cases of moderately severe tissue injury were observed, occurring in the high concentration exposure group in olfactory tissue. In addition, all animals survived to the end of the study and the treatment-related labored breathing and nasal discharge generally decreased in incidence during the second half of the study.

We did not apply an interspecies toxicokinetic $UF_{A\text{-}k}$. Hybrid computational fluid dynamics and PBPK modeling for predicting nasal tissue dose metrics show that the predicted dose to the epithelium of the total nasal cavity following inhalation of

an organic gas is similar, or slightly greater, in humans compared to rats (Frederick et al., 2001). Also, injury occurred to regions of the upper respiratory tract that are most sensitive to both chemical and mechanical irritants (primarily due to airflow characteristics) which indicates that caprolactam is primarily a direct-acting irritant, rather than a chemical requiring metabolic activation in nasal mucosa to cause tissue injury (Kilgour et al., 2000; Harkema et al., 2006; Renne et al., 2007; Kaufmann et al., 2009). In particular, Kaufmann et al. (2009) indicated the rat larynx may be more sensitive to inhaled irritant gases due to air flow characteristics than other species such as humans, monkeys and dogs. Therefore, the human equivalency concentration (HEC) adjustment for upper respiratory tract injury should also be sufficient for any residual interspecies toxicokinetics differences.

We applied a default interspecies UF_{A-d} of $\sqrt{10}$ to compensate for the absence of data on pharmacodynamic differences between species. Specifically, only one comprehensive animal inhalation study in rats has been performed with caprolactam.

The toxicokinetic data for inspired upper respiratory irritants in humans suggest low interindividual variation and no dosimetry differences between adults and children (OEHHA, 2008). Thus, no UF_{H-k} is applied for intraspecies toxicokinetic variation among individuals.

While caprolactam is irritating to the upper respiratory tract, initiation or exacerbation of asthma by caprolactam has not been characterized. However, data summarized by OEHHA (2008) show asthmatics may be more sensitive to the effects of respiratory irritants than non-asthmatic individuals. Thus, an intraspecies toxicodynamic UF of 10 is applied to address the diversity in the human population, including children with asthma. Of equal concern are the consistent findings of seizures in heavily exposed adult workers and in experimental animal data. Children may be more sensitive than adults to chemicals that have neurological effects, and the finding of neurotoxicity in workers additionally supports the application of a 10-fold intraspecies toxicodynamic UF. Application of the cumulative UF = 60 to the human equivalent concentration of 0.402 mg/m^3 resulted in an 8-hour REL of 7 $\mu g/m^3$ (1.4 ppb) for caprolactam.

8.3 Derivation of the Chronic Inhalation Reference Exposure Level

Study	Reinhold et al. 1998
Study population	Sprague-Dawley CD rats (10 animals/sex/group)
Exposure method	Discontinuous whole-body inhalation exposure to 0, 24, 70, and 243 mg/m^3 caprolactam aerosol
Critical effects	Upper airway lesions of nasal and laryngeal epithelium
LOAEL	24 mg/m^3
NOAEL	Not observed
$BMCL_{05}$	3 mg/m^3
Exposure continuity	6 hours per day, 5 days/week
Exposure duration	13 weeks
Average experimental exposure	0.536 mg/m^3 (3 mg/m^3 x 6/24 hr x 5/7 days)
Human equivalent concentration	0.134 mg/m^3 (for extrathoracic respiratory effects, RGDR = 0.25
LOAEL uncertainty factor	1 ($BMCL_{05}$ used as point of departure)
Subchronic uncertainty factor	2 (for13 wk exposure in rodents)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1
Toxicodynamic (UF_{A-d})	√10 (default: no toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (site of contact; no systemic effects)
Toxicodynamic (UF_{H-d})	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	60
Chronic reference exposure level	2.2 µg/m^3 (0.5 ppb)

The chronic REL is based on the same study as the 8-hr REL. The chronic REL derivation is the same as that used for the 8-hr REL, with the exception that the average experimental exposure is based on continuous, 24 hr/day exposure. The resulting human equivalent concentration is reduced to 0.134 mg/m^3. The application of uncertainty factors was the same for both 8-hr and chronic RELs, resulting in a chronic REL of 2.2 µg/m^3 (rounded to two significant figures from 2.23 µg/m^3 to avoid rounding errors because the first digit is a 2), or 0.5 ppb (rounded to one significant figure from 0.48 ppb).

8.4 Data Strengths and Limitations for Development of the REL

Significant strengths for the caprolactam RELs include: (1) the use of a well-conducted subchronic animal study with histopathological analysis, and (2) independent studies demonstrating comparable key irritant effects (nasal and throat irritation) in humans and experimental animals. Major areas of uncertainty are: (1) the lack of comprehensive human inhalation dose-response data for long-

term exposures, (2) no inhalation developmental/reproduction toxicity data, although sufficient oral developmental/reproduction data exist (However, when converted to an air concentration, the level that causes fetotoxicity is greater than the level that results in severe pulmonary injury), (3) the absence of a NOAEL in the subchronic study, and (4) no chronic animal inhalation exposure studies.

9. References

ACGIH. (2001). Caprolactam. American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio. Online at: www.acgih.org.
ACGIH. (2003). Caprolactam. American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio. Online at: www.acgih.org.
Aguirre A, Gonzalez Perez R, Zubizarreta J, Landa N, Sanz de Galdeano C and Diaz Perez JL (1995). Allergic contact dermatitis from epsilon-caprolactam. Contact Dermatitis 32(3): 174-5.
Ballistreri A, Garazzo D, Giuffrida M, Montaudo G, Filippi A, Guaita C, Manaresi P and Pilati F (1987). Fast atom bombardment mass spectrometry identification of oligomers contained in poly(epsilon-caprolactam) and poly(butylene isophthalate). Macromolecules 20: 1029-32.
BASF. (1985). Test report: Acute inhalation toxicity LC_{50}, 4 hours (rat): Evaluation of caprolactam (compound no. 84/217) as an aerosol liquid. Submitted by BASF Corporation, Parsippany, NJ, under TSCA Section 8(d) November 26, 1990
Billmaier DJ, Knowlden NF and Stidham DW (1992). Caprolactam: A study of current workers. Allied-Signal Inc., Morristown, NJ. EPA/OTS Doc #86-920001041. 1-16, A-1-A-9.
Bongard W (2000). Competitiveness of Nylon 6. Man-Made Fiber Year Book DSM Fibre Intermediates, Geleen, Netherlands: 14-15.
Bonifaci L, Frezzotti D, Cavalca G, Malauti E and Ravanetti GP (1991). Analysis of epsilon-caprolactam and its oligomers by high-performance liquid chromatography. J Chromatog 585: 333-36.
Bradley EL, Speck DR, Read WA and Castle L (2004). Method of test and survey of caprolactam migration into foods packaged in nylon-6. Food Addit Contam 21(12): 1179-85.
Burdock GA, Kulwich BA, Alsaker RD and Marshall PM (1984). Ninety Day Toxicity Study in Dogs with Caprolactam, Hazelton Laboratories America, Vienna, VA. In: Proceedings of a Symposium on an Industry Approach to Chemical Risk Assessment - Caprolactam and Related Compounds as a Case Study, May 15-17, 1984, Arlington VA. pp. 97-113. Hazelton Laboratories America, Vienna, VA.
Cain WS, Lee NS, Wise PM, Schmidt R, Ahn BH, Cometto-Muniz JE and Abraham MH (2006). Chemesthesis from volatile organic compounds: Psychophysical and neural responses. Physiol Behav 88(4-5): 317-24.
Cal/OSHA. (2011). Permissable Exposure Limits for Chemical Contaminants. California Occupational Safety and Health Administration, Online at: www.dir.ca.gov/title8/5155table_ac1.html.
Chen H (2002). A caprolactam poisoning report [in Chinese]. China Occup Med 29(2): 61.

Cheng Y, Li SM and Leithead A (2006). Chemical characteristics and origins of nitrogen-containing organic compounds in PM2.5 aerosols in the Lower Fraser Valley. Environ Sci Technol 40(19): 5846-52.
Cooper JE, Cooke DP, Dickson WA, Noell LT, Beal BM and Pirtle LS (1993). Petition to Delist Caprolactam Under Section 112(b) of the Clean Air Act. AlliedSignal Inc. Properties, Standards, and Use of Caprolactam. BASF Corporation, DSM Chemicals North America Inc. pp. 2-1 to 2-7.
CPSC. (1996). Sensory and Pulmonary Irritation Studies of Carpet System Materials and their Constituent Chemicals U.S. Consumer Product Safety Commission. Contract CPSC-C-94-1122. AQS Report No. 01890-06. Chapters 1 to 5 available at Internet address:http://www.cpsc.gov/LIBRARY/FOIA/FOIA98/os3519926D.pdf. Chapters 6 through Appendix B available at http://www.cpsc.gov/LIBRARY/FOIA/FOIA98/os/3512B0C.pdf.
Dalton P (2003). Upper airway irritation, odor perception and health risk due to airborne chemicals. Toxicology letters 140-141: 239-48.
Ferguson WS and Wheeler DD (1973). Caprolactam vapor exposures. Am Ind Hyg Assoc J 34(9): 384-9.
Frederick CB, Gentry PR, Bush ML, Lomax LG, Black KA, Finch L, Kimbell JS, Morgan KT, Subramaniam RP, Morris JB and Ultman JS (2001). A hybrid computational fluid dynamics and physiologically based pharmacokinetic model for comparison of predicted tissue concentrations of acrylic acid and other vapors in the rat and human nasal cavities following inhalation exposure. Inhal Toxicol 13(5): 359-76.
Gad SC, Robinson K, Serota DG and Colpean BR (1987). Developmental toxicity studies of caprolactam in the rat and rabbit. J Appl Toxicol 7(5): 317-26.
Gill K (2011). Written communication. Air Resources Board, California Environmental Protection Agency, Sacramento, CA.
Goldblatt MW, Farquharson ME, Bennett G and Askew BM (1954). Epsilon-Caprolactam. Br J Ind Med 11(1): 1-10.
Gross P (1984). Biologic activity of epsilon-caprolactam. Crit Rev Toxicol 13(3): 205-16.
Harkema JR, Carey SA and Wagner JG (2006). The nose revisited: a brief review of the comparative structure, function, and toxicologic pathology of the nasal epithelium. Toxicol Pathol 34(3): 252-69.
Haskell Laboratory (1950). Medical Research Project No. MR-173: Toxicity of Epsilon-Caprolactam. Report # HL-0022-50, Haskell Laboratory of Industrial Toxicology, Wilmington, Delaware.
Hausen BM (2003). Allergic contact dermatitis from colored surgical suture material: contact allergy to epsilon-caprolactam and acid blue 158. Am J Contact Dermat 14(3): 174-5.
Hegde RR, Dahiya A and Kamath MG (2004). Nylon Fibers. Online at: www.engr.utk.edu/mse/Textiles/Nylon%20fibers.htm.
Hodgson AT, Shendell DG, Fisk WJ and Apte MG (2004). Comparison of predicted and derived measures of volatile organic compounds inside four new relocatable classrooms. Indoor Air 14 Suppl 8: 135-44.

Hoffman GM. (1997). A 13-week inhalation toxicity study (with a 4-week recovery) of caprolactam (494-95A) in the rat via whole-body exposures. Huntingdon Life Sciences, East Millstone, NJ, Volume I to III, Study no.: 95-6095, IHF 3-95PA.
Hohensee F (1951). On the pharmacological and physiological effects of e-caprolactam. Faserforsch Textultech 2: 299-303.
HSDB (2006). Hazardous Substances Data Bank. Chemical/Physical Properties; Emergency Medical Treatment. National Library of Medicine, Bethesda, MD. Available at: http://toxnet.nlm.nih.gov.
IARC (1999). Caprolactam. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Re-evalualtion of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide. World Health Organization, Lyon, France. 71 (part 2): 383-400.
IWMB (2003). Integrated Waste Management Board. Building Material Emissions Study. Sacramento, CA. Available at: www.ciwmb.ca.gov/Publications. pp. 1-15, 34-37, 62-63, 90-92, 164-184 (App. C), 305-308 (App. O).
Kaufmann W, Bader R, Ernst H, Harada T, Hardisty J, Kittel B, Kolling A, Pino M, Renne R, Rittinghausen S, Schulte A, Wohrmann T and Rosenbruch M (2009). 1st international ESTP expert workshop: "Larynx squamous metaplasia". A re-consideration of morphology and diagnostic approaches in rodent studies and its relevance for human risk assessment. Exp Toxicol Pathol 61(6): 591-603.
Kelman GR (1986). Effects of human exposure to atmospheric epsilon-caprolactam. Hum Toxicol 5(1): 57-9.
Kilgour JD, Simpson SA, Alexander DJ and Reed CJ (2000). A rat nasal epithelial model for predicting upper respiratory tract toxicity: in vivo-in vitro correlations. Toxicology 145(1): 39-49.
Kirk LK, Lewis BA, Ross DA and Morrison MA (1987). Identification of ninhydrin-positive caprolactam metabolites in the rat. Food Chem Toxicol 25(3): 233-239.
Krajnik V, Bozek P, Kondelikova J and Kralicek J (1982). High-performance liquid chromatography of 6-caprolactam and its cyclic oligomers present in polyamide 6. J Chromatog 240: 539-42.
Lan X-x, Bai T-j, Yang Z-r and Luo K-z (1998). Health status of workers exposed to caprolactam [in Chinese]. J China Ind Med 11(2): 115.
Lander J-A. (2002). Structure Development in Silicate-Layered Polymer Nanocomposites. A Thesis Submitted for the Degree of Doctor of Philosophy. Uxbridge, Middlesex: Wolfson Centre for Materials Processing, Brunel University.
Lang I, Bruckner T and Triebig G (2008). Formaldehyde and chemosensory irritation in humans: a controlled human exposure study. Regulatory toxicology and pharmacology : RTP 50(1): 23-36.
Li J (1996). Study of health effects of caprolactam on exposed workers [in Chinese]. Ind Hyg Occup Dis 22(2): 103-04.
Liu F-q, Bao Y-s and Zheng C-l (1988). Investigation on the effects of caprolactam on the maternal functions of female workers [in Chinese]. Chinese Journal of Industrial Hygiene and Occupational Diseases 6(2): 201-3.
Morgan KT (1991). Approaches to the identification and recording of nasal lesions in toxicology studies. Toxicol Pathol 19(4 Pt 1): 337-51.

Nau DR, Darr RW, Gad SC and Pai SV (1984). Validation Study of a Method for Monitoring Personnel Exposure to Caprolactam. In: Proceedings of a Symposium on an Industry Approach to Chemical Risk Assessment - Caprolactam and Related Compounds as a Case Study, May 15-17, 1984, Arlington VA. pp. 97-113.
Nielsen GD, Wolkoff P and Alarie Y (2007). Sensory irritation: risk assessment approaches. Regul Toxicol Pharmacol 48(1): 6-18.
NIOSH. (1995a). Occupational Safety and Health Guideline for Caprolactam Dust. National Institute for Occupational Safety and Health, U.S. Department of Health and Human Services.
NIOSH. (1995b). Occupational Safety and Health Guideline for Caprolactam Vapor. National Institute for Occupational Safety and Health, U.S. Department of Health and Human Services.
NPG (2007). Nylon-6. Nylon-6 Promotional Group, Online at: www.npg-6.com.
NTP (1982). Carcinogenesis bioassay of caprolactam (CAS No. 105-60-2) in F344 rats and B6C3F$_1$ mice (feed study). National Toxicology Program, Technical Report Series No. 214. Online at: http://ntp-server.niehs.nih.gov.
OEHHA. (2008). Air Toxics Hot Spots Program Risk Assessment Guidelines. Technical Support Document for the Derivation of Noncancer Reference Exposure Levels. California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Oakland, CA. Online at: http://www.oehha.ca.gov/air/hot_spots/rels_dec2008.html.
OSHA. (1988). Caprolactam. Related Information: Chemical Sampling - Caprolactam (Dust), Caprolactam (Vapor). Occupational Safety and Health Administration, U.S. Department of Labor. Online at: http://www.osha.gov/dts/sltc/methods/partial/pv2012/2012.html.
Osimitz TG, Droege W and Finch JM (2007). Toxicologic significance of histologic change in the larynx of the rat following inhalation exposure: a critical review. Toxicol Appl Pharmacol 225(3): 229-37.
Reinhold RW, Hoffman GM, Bolte HF, Rinehart WE, Rusch GM, Parod RJ and Kayser M (1998). Subchronic inhalation toxicity study of caprolactam (with a 4-week recovery) in the rat via whole-body exposures. Toxicol Sci 44(2): 197-205.
Renne R, Brix A, Harkema J, Herbert R, Kittel B, Lewis D, March T, Nagano K, Pino M, Rittinghausen S, Rosenbruch M, Tellier P and Wohrmann T (2009). Proliferative and nonproliferative lesions of the rat and mouse respiratory tract. Toxicol Pathol 37(7 Suppl): 5S-73S.
Renne RA and Gideon KM (2006). Types and patterns of response in the larynx following inhalation. Toxicol Pathol 34(3): 281-5.
Renne RA, Gideon KM, Harbo SJ, Staska LM and Grumbein SL (2007). Upper respiratory tract lesions in inhalation toxicology. Toxicol Pathol 35(1): 163-9.
Riedl O, Hruskova J, Stuchlikova E, Komarkova A, Novotna Blazkova B, Tenorova M, Renner J, Spala M and Tvaroh F (1963). Treatment of obesity with caprolactam. Rev Czech Med 24: 167-82.
Rinehart WE, Alarie Y, Stock MF and Rush RE (1997). The sensitization potential of caprolactam. Int J Toxicol 16 (suppl 2): 22.

Ritz J, Fuchs H, Kieczka H and Moran WC (2002). Caprolactam. In: Ullmann's Encyclopedia of Industrial Chemistry. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. New York: 1-21.
Salamone MF (1989). Abnormal sperm assay tests on benzoin and caprolactam. Mutat Res 224(3): 385-9.
Serota DG, Hoberman AM, Friedman MA and Gad SC (1988). Three-generation reproduction study with caprolactam in rats. J Appl Toxicol 8(4): 285-93.
Springborn Laboratories Inc. (1991). Life Sciences Division, SLS Study No. 3224.7, April 25, 1991.
Stuart BO (1984). Deposition and clearance of inhaled particles. Environ Health Perspect 55: 369-90.
Swift DL and Strong JC (1996). Nasal deposition of ultrafine ^{218}Po aerosols in human subjects. J Aerosol Sci 27(7): 1125-32.
Tuma SN, Orson F, Fossella FV and Waidhofer W (1981). Seizures and dermatitis after exposure to caprolactam. Arch Intern Med 141(11): 1544-5.
U. S. EPA. (1994a). Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. U.S. Environmental Protection Agency, EPA/600/8-90/066F.
U. S. EPA (1994b). Caprolactam (CASRN 105-60-2). U. S. Envoronmental Protection Agency. Integrated Risk Information System. Available at: http://www.epa.gov/iris. pp.1-13.
U. S. EPA. (2006). Inventory Update Reporting. Non-confidential 2006 IUR Records by Chemical, including Manufacturing, Processing and Use Information. Online at: http://cfpub.epa.gov/iursearch/2006_iur_natlcheminfo.cfm?id=2673.
U. S. EPA. (2009a). Epsilon-Caprolactam (CAS # 105-60-2). Letter of test results performed in 1952-53 filed with Office of Pollution Prevention and Toxics 8(e) coordinator, U.S. Environmental Protection Agency, Washington, DC, 8EHQ-09-17699, dated October 28, 2009.
U. S. EPA. (2009b). Metabolically derived human ventilation rates: A revised approach based upon oxygen consumption rates. U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC; EPA/600/R-06/129F.
U. S. EPA. (2009c). Benchmark Dose Software, Version 2.1.1. National Center for Environmental Assessment. Online at: http://www.epa.gov/ncea/bmds.
Unger PD, Salerno AJ and Friedman MA (1981). Disposition of 14C-caprolactam in the rat. Food Cosmet Toxicol 19: 457-462.
Venema A, Henk van de Ven JFM, David F and Sandra P (1993). Supercritical fluid extraction of Nylon 6: An investigation into the factors affecting the efficiency of extraction of caprolactam and its oligomers. J High Resolution Chromatography 16: 522-24.
Vihola H, Laukkanen A, Valtola L, Tenhu H and Hirvonen J (2005). Cytotoxicity of thermosensitive polymers poly(N-isopropylacrylamide), poly(N-vinylcaprolactam) and amphiphilically modified poly(N-vinylcaprolactam). Biomaterials 26: 3055-64.
Waddell WJ, Marlowe C and Friedman MA (1984). The distribution of [^{14}C]caprolactam in male, female, and pregnant mice. Food Chem Toxicol 22(4): 293-303.

Walker JC, Kendal-Reed M, Utell MJ and Cain WS (2001). Human breathing and eye blink rate responses to airborne chemicals. Environ Health Perspect 109 Suppl 4: 507-12.
Wilke O, Jann O and Brodner D (2004). VOC- and SVOC-emissions from adhesives, floor coverings and complete floor structures. Indoor Air 14 Suppl 8: 98-107.
Wilkins CK, Wolkoff P, Gyntelberg F, Skov P and Valbjorn O (1993). Characterization of office dust by VOCs and TVOC release - Identification of potential irritant VOCs by partial least squares analysis. Indoor Air 3: 283-290.
Woo KH, Jung SJ, Park WS, Shin HR, Kim JS and Kim SW (1998). Two cases of convulsion associated with caprolactam [in Korean]. Korean J Occup Environ Med 10(1): 116-20.
Working PK (1989). Assessment of unscheduled DNA synthesis in Fischer 344 rat pachytene spermatocytes exposed to caprolactam or benzoin in vivo. Mutat Res 224(3): 365-8.
Xu Y-p, Ma B-l, Wang X-s, Wang M-s, Zhang L and Li Z (1997). Effects of low concentration caprolactam on worker health [in Chinese]. Chinese Journal of Industrial Medicine 10(5): 290-92.
Yeh H-C, Cuddihy RG, Phalen RF and Chang I-Y (1996). Comparisons of calculated respiratory tract deposition of particles based on the proposed NCRP model and the new ICRP66 model. Aerosol Sci Technol 25: 134-40.
Zaitsau DH, Paulechka YU, Kabo GJ, Kolpikau AN, Emel'yanenko VN, Heintz A and Verevkin SP (2006). Thermodynamics of the sublimation and of the vaporization of e-caprolactam. J Chem Eng Data 51: 130-35.
Zhou H-d (1976). Occupational poisoning tests (manual) [in Chinese]. People's Medical Publishing House, Beijing: 424-6.
Ziegler AE, Zimmer H and Triebig G (2008). Exposure study on chemosensory effects of epsilon-caprolactam in the low concentration range. Int Arch Occup Environ Health 81(6): 743-53.

Appendix A

Benchmark Concentration (BMC) Modeling of the Upper Respiratory Tract Lesions Resulting from Subchronic Caprolactam Exposure in Rats (Reinhold et al., 1998)

Pathologist-graded lesions in the nasal and laryngeal airways provided dose-response data for BMC modeling. Males and females were combined, given that no apparent gender differences were noted from the endpoint responses. US EPA does not as yet recommend an approved protocol for modeling categorical data such as lesion grades. So the Reinhold data were modeled as dichotomous data (i.e., all lesions of severity categories found in both control and treated animals were designated as not treatment-related; all lesions of severity categories found in treated animals that were greater than that found in control animals were designated as a treatment-related effect).

Per US EPA protocol, P-values ≤ 0.1 indicate that the model is a poor fit to the data and recommended not to be used for BMC determination. US EPA also states that P-values identify those models that are consistent with the experimental results, but should not be compared among the various models. The Akaike Information Criterion (AIC) can be used to compare models. Generally, the model with the lowest AIC is considered the preferential model, although other considerations such as model fit to the low end of the concentration curve where the $BMCL_{05}$ resides are also taken into account. Three regions of the upper respiratory tract (nasal olfactory mucosa, nasal respiratory mucosa and laryngeal epithelial tissue) at sacrifice provided dose response data on which to run BMC modeling:

1) **Incidence of epithelial intracytoplasmic eosinophilic material in nasal olfactory mucosa**

Minimal-grade lesions were present in most animals in the control group. Caprolactam exposed animals exhibited slight, moderate and moderately severe lesions, which we considered exposure-related. The P-values and AIC indicate the log-probit and log-logistic models show the best fit to the data, particularly at the low end of the dose-response curve where the BMC_{05} lies (models highlighted in bold in Table A-1). The BMC_{05} are nearly identical for these two models but the log-probit has a smaller range between the MLE and the BMC_{05} suggesting greater confidence with this model at the low concentration end where the $BMCL_{05}$ resides, even though the log-logistic model fit produces a slightly smaller AIC. Thus, the log-probit model was chosen as the basis for the point of departure for this endpoint.

Table A-1. Benchmark concentration results modeling incidence (slight, moderate and moderately severe grades combined) of epithelium-intracytoplasmic eosinophilic material in nasal olfactory mucosa.

Method	$BMCL_{05}$	BMC_{05}	P-value	AIC
Probit	18	25	0.11	66.457
Log-probit (slope restricted ≥ 1)	**12**	**17**	**0.99**	**60.845**
Logistic	18	26	0.097	66.925
Log-logistic(slope restricted ≥ 1)	**5.6**	**16**	**0.99**	**60.834**
Weibull (power ≥ 1)	5.3	11	0.90	61.038
Gamma (power ≥ 1)	5.3	12	0.93	60.984
Quantal-linear	5.2	7.2	0.90	59.495
Quantal-quadratic	29	36	0.055	67.148
Multistage (2nd degree)	5.3	8.8	0.85	61.180

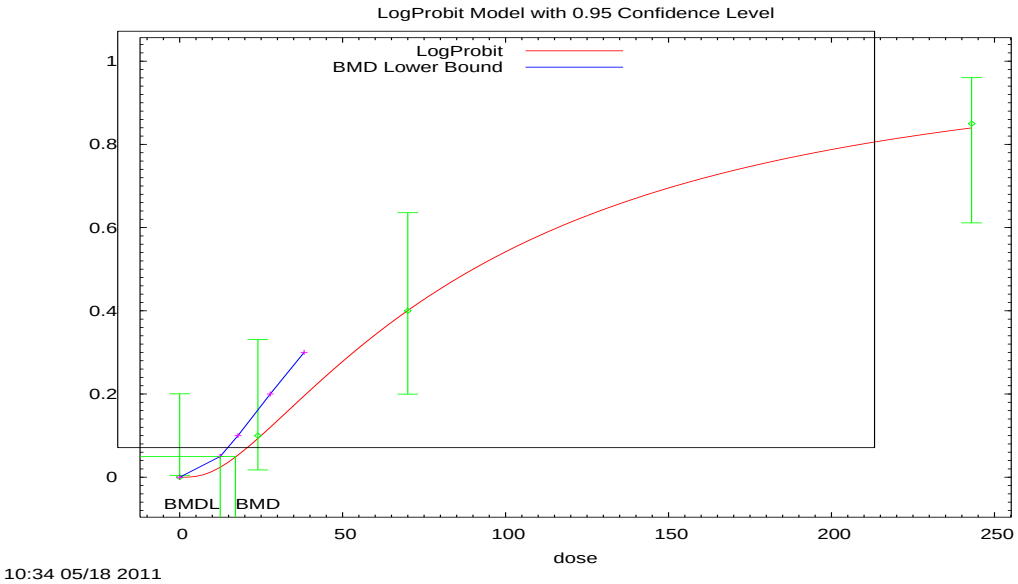


Figure A-1: Log-probit model fit to nasal olfactory mucosa lesion incidence data

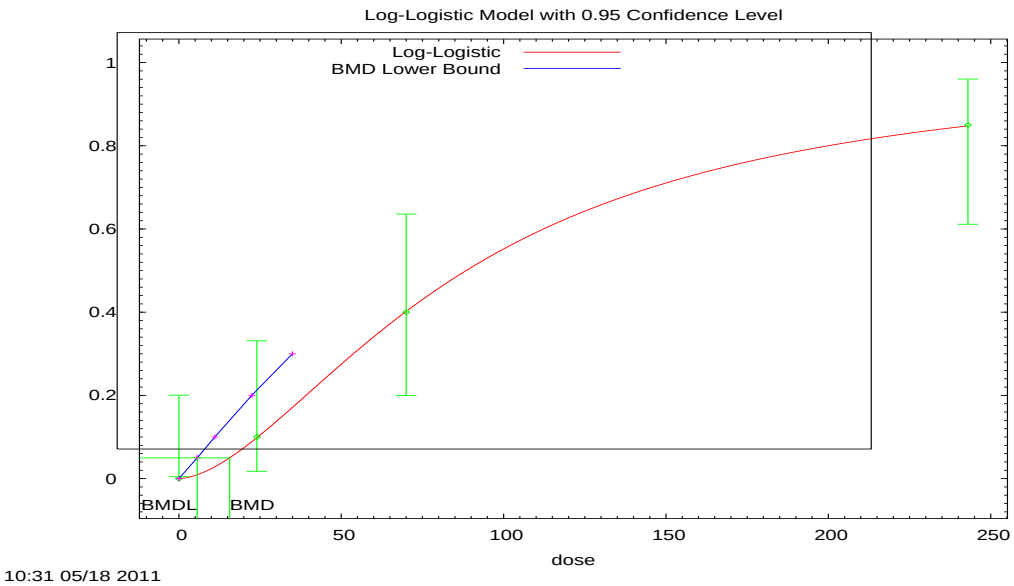


Figure A-2: Log-logistic model fit to nasal olfactory mucosa lesion incidence data

2) Incidence of goblet cell hypertrophy/hyperplasia in respiratory nasal mucosa

Minimal and slight lesions of the nasal respiratory epithelium were present in control animals. Moderate-grade lesions were present in caprolactam-exposed animals and considered exposure-related. Models in Table A-2 with P-values <0.10 are not considered a good fit to the data. Among the remaining models, the log-logistic model (shown in bold in Table A-2) had the lowest AIC and best fit to the data, thus we chose the $BMCL_{05}$ from the log-logistic model as the point of departure for this endpoint.

Table A-2: BMC results modeling moderate-grade incidence of goblet cell hypertrophy/hyperplasia in respiratory nasal mucosa.

Method	$BMCL_{05}$	BMC_{05}	P-value	AIC
Probit	21	29	0.024	88.321
Log-probit (slope restricted ≥ 1)	15	24	0.026	86.384
Logistic	22	31	0.022	88.643
Log-logistic (slope restricted ≥ 1)	**3.6**	**5.9**	**0.78**	**77.525**
Weibull (power ≥ 1)	6.8	9.4	0.21	80.650
Gamma (power ≥ 1)	6.8	9.4	0.21	80.650
Quantal-linear	6.8	9.4	0.21	80.650
Quantal-quadratic	45	59	0.017	88.507
Multistage (2nd degree)	6.8	9.4	0.21	80.650

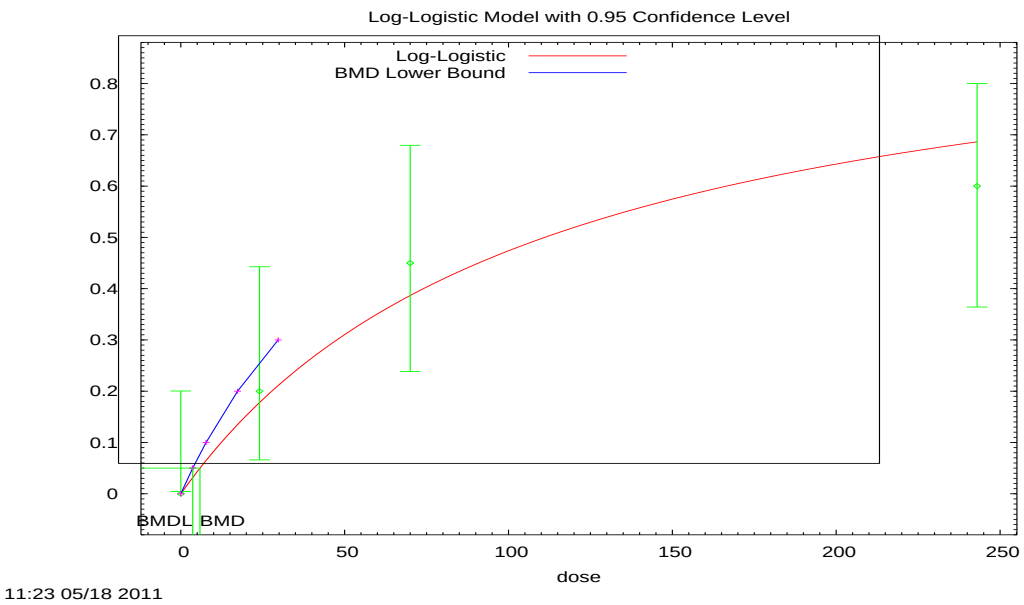


Figure A-3: Log-logistic model fit to nasal respiratory mucosa lesion incidence data

3) Incidence of squamous/squamid metaplasia/hyperplasia of laryngeal tissue

Minimal and slight severity levels present in caprolactam-exposed animals were considered exposure-related. The model that provided a low AIC, best p-value and best fit to the data at the low concentration end of the curve was the multistage model (Table A-3). Although the quantal-linear model produced a lower AIC, the line fit to the data point at the low end of the curve (24 mg/m^3) was not quite as good as that provided by the multistage model (Figures A-4 and A-5). Thus, we chose the multistage model as the basis for the point of departure for this endpoint.

Table 3. Benchmark concentration results modeling incidence of (minimal and slight combined) squamous/squamid metaplasia/hyperplasia of laryngeal tissue

Method	$BMCL_{05}$	BMC_{05}	P-value	AIC
Probit	8.3	12.6	0.33	56.43
Log-probit (slope restricted ≥ 1)	6.7	11.9	0.52	55.20
Logistic	8.9	13.6	0.29	56.92
Log-logistic (slope restricted ≥ 1)	3.9	10.7	0.44	55.80
Weibull (power ≥ 1)	2.7	7.2	0.84	53.85
Gamma (power ≥ 1)	2.7	7.8	0.79	54.06
Quantal-linear	**2.6**	**3.6**	**0.82**	**52.92**
Quantal-quadratic	12.2	14.9	0.33	54.27
Multistage (2nd degree)	**2.8**	**5.3**	**0.94**	**53.59**

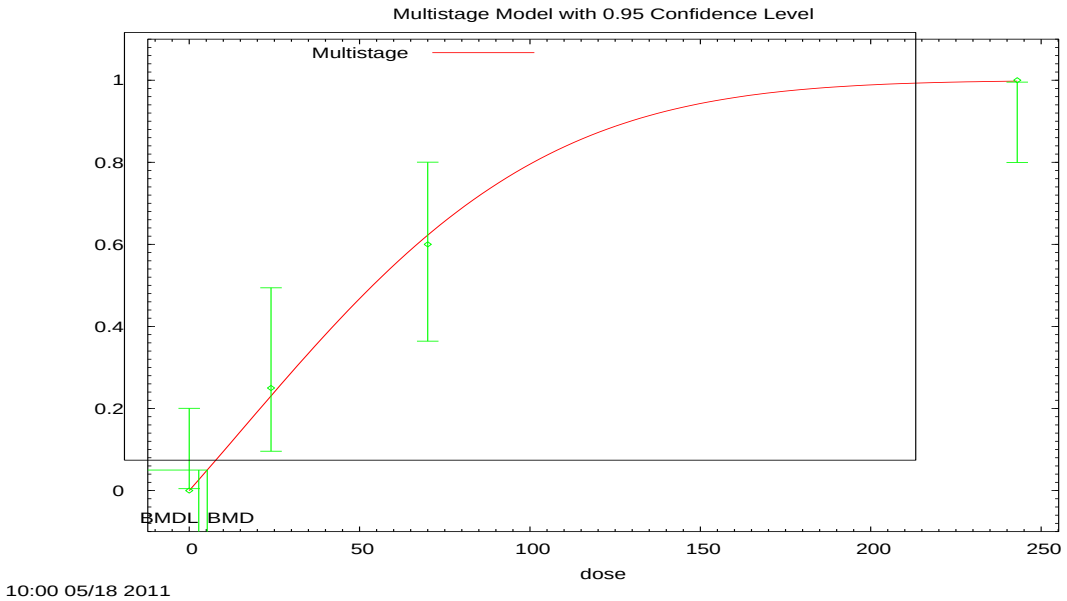


Figure A-4: Multistage model fit to laryngeal lesion incidence data

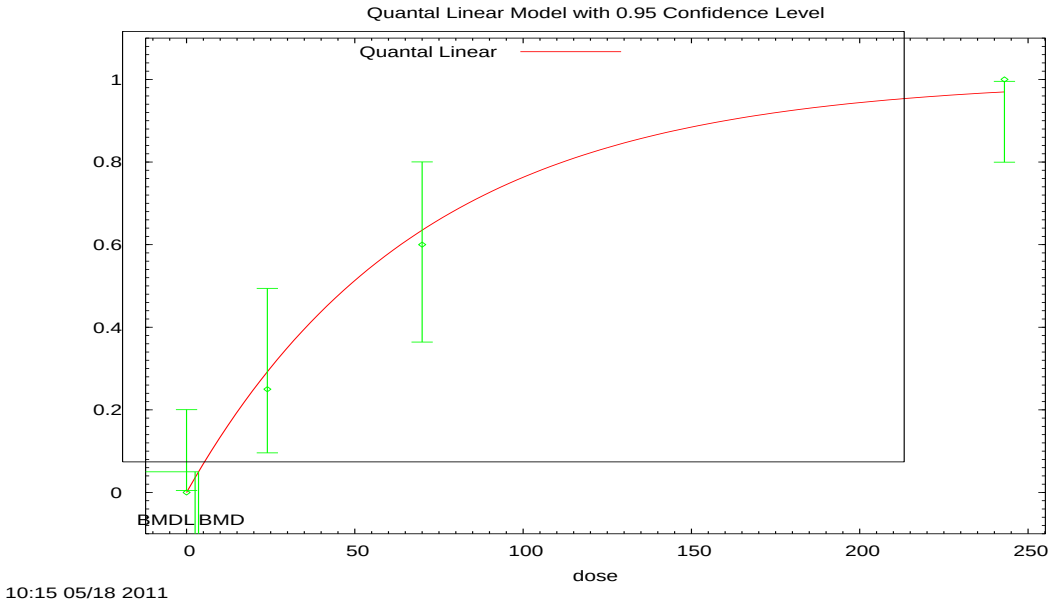


Figure A-5: Quantal linear model fit to laryngeal lesion incidence data