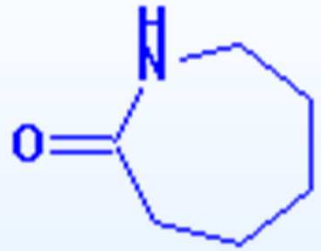
Caprolactam
Reference Exposure Levels

Scientific Review Panel Meeting

October 31, 2011

Office of Environmental Health Hazard Assessment

Caprolactam
Preceding SRP Meeting



- **At the May 2011 SRP meeting:**
  - **Presented a draft 8-hr REL (7 µg/m^3) and a chronic REL (2.2 µg/m^3)**
  - **Based on subchronic exposure study in rats – lesions in nasal and larynx epithelium**
  - **No acute REL – attempting to get raw data from acute human study**

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Overview of Changes to the Document

- **Derived acute REL (50 µg/m^3) based on OEHHA analysis of raw data from Ziegler study**

Added in response to Panel:

- **Appendix of detailed benchmark concentration modeling results of Reinhold 13-week rat data**
- **Chinese and Korean case reports of neurotoxicity with heavy worker exposure**
- **Summaries of Chinese caprolactam occupational studies**
- **Case reports of contact dermatitis resulting from dermal exposure**

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Overview of Changes (Continued)

Added:

- **Summary tables of acute and chronic exposure results in animals and humans**
- **Tables to clarify the results from the rat 13-week exposure study:**
  - **Daily and weekly observation findings**
  - **Modified table to present results of 13-week exposure, and 13-week exposure + 4-week recovery**

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OEHHA Analysis of Acute Raw Data

Human Chamber study (Ziegler et al. 2008)

- Exposure: 0, 0.15, 0.5, 5 mg/m^3 , n=20
- Measures at 0 (just after entering chamber), 1, 3, and 6 hrs of exposure
  - Eye blink frequency
  - Eye redness
  - Nasal resistance (measured at 6 hrs only)
  - Subjective symptom questionnaire

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Tests Applied to Acute Raw Data

Page's Trend Test used for statistical analysis

- Applied to non-normally distributed data
- Takes into account measurement of same subjects at different exposure times
- Takes the ordering of the doses into account
- If trend $p \leq 0.05$, Wilcoxon sign-rank test used to find dose group differences

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OEHHA Analysis of Acute Raw Data

Objective measures at 1 hour of exposure

- No statistically significant trend at $p<0.05$ for eye redness and nasal resistance
- Statistical significance for eye blink frequency
  - Page's trend test: $p=0.002$ at 1 hr
  - Difference from control, Wilcoxon sign-rank test: $p=0.013$ for 5 mg/m^3

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OEHHA Analysis of Acute Raw Data

Subjective Symptom Results at 1 hour

- 29 questions placed in 7 subgroups
- Statistically significant trend for eye irritation
  - Page's trend test: ***p*=0.025**
  - Difference from control, Wilcoxon sign-rank test: ***p*=0.016 for 5 mg/m^3**
- No statistically significant trend or difference from controls for nasal irritation

Caprolactam
Acute REL Derivation

Critical Effect: Increased eye blink frequency

- **LOAEL: 5 mg/m^3**
- **NOAEL: 0.5 mg/m^3 (POD)**
- **No time adjustment – 1 hr exposure data**
- **No interspecies adjustment – human data**
- **Intraspecies UFs:**
  - **toxicokinetic $UF_{H\text{-}k}$ = 1 (site of contact irritant)**
  - **toxicodynamic $UF_{H\text{-}d}$ = 10 (for human variation)**
- **Cumulative UF = 10**
- **Acute REL = 50 µg/m^3 (11 ppb)**

New Caprolactam Reports
Added a Chinese & Korean Case Report

- **Heavy exposure led to seizures in workers**
- **Supports Tuma report of seizures with heavy caprolactam exposure**
- **Supports use of Intraspecies UF=10 for child sensitivity to neurotoxicants**

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Added Chinese Occuptional Studies

- **Four studies translated from Chinese to English**
- **Symptoms included dizziness, insomnia, nausea, nosebleed, dermal lesions, nasal symptoms (i.e., dryness, rhinitis, sinusitis) & dysmenorrhea, primary infertility, pregnancy hypertension in female workers**
- **Methodology and results too brief and lacked details for basis of chronic REL**

Stakeholder Comments

Material Sent to Panel

- **The Panel members received material from industry stakeholders in the last several weeks**
- **Much of the material reiterated comments received during the public comment period, which were already addressed by OEHHA**
- **We provide commentary in the next several slides on new comments and points, primarily concerning the draft acute REL.**

Comments Regarding OEHHA Statistics for Basis of the Acute REL

OEHHA is “cherry-picking” from the raw eye blink data to show a statistically significant increase in blink frequency:

Used 1 hr data from manual “lights-off” approach that was statistically significant and ignored 1 hr data using the semi-automated “lights-on” approach that was not statistically significant.

Response: Both eye blink recording methods show statistically significant increased trend in blink rate, although not at all time points

Zeigler indicates “lights-on” method not fully vetted.

Statistical Significance of Eye Blink Data

	Eye Blink Analysis Method	
Time Point During Exposure	**Traditional dim light (Lights-off) manual count method**	**Semi-automated neon light (Lights-on) method**
0 Hr	Page's: p = 0.88	Page's: p = 0.13
1 Hr	Page's: p = 0.002	Page's: p = 0.230
3 Hr	Not enough data	Page's: p = 0.01
6 Hr	Not enough data	Page's: p = 0.0001
All 4 time points combined	Not enough data	Page's: p = 0.022

Comments Regarding OEHHA Statistics for Basis of the Acute REL

Prefer Page's trend test because it takes the ordering of the doses into account

The Friedman's test (a nonparametric two-way ANOVA) ignores the ordering of the doses, and the repeated measures ANOVA assumes normality and also ignores the ordering of doses

Response: OEHHA agrees with recommendation and proposes to the Panel that only the statistical analysis with the Page's trend test be presented in the Final REL document.

Comment: “Day Effect” as a Confounding Factor

Eye blink data suggests subjects become increasingly familiar with tests during week of exposures resulting in increased blink frequency on successive days testing (i.e., the “day effect”).

Exposure study design was “unbalanced”, leading to confounding “day effect”.

Study Design Used by Ziegler

Order of Dose (mg/m^3) Testing By Week of Experiment

Exposure week	Day 1	Day 2	Day 3	Day 4
1	0	0.5	0.15	5.0
2	5.0	0.5	0.15	0
3	0.5	0	0.15	5.0
4	0.15	0	5.0	0.5
5	0	0.15	0.5	5.0

"Day Effect"
Study Design Proposed by Dr. Haseman

	Order of Dose (mg/m^3) Testing By Week of Experiment			
Exposure week	Day 1	Day 2	Day 3	Day 4
1	0	0.5	0.15	5.0
2	0.15	5.0	0.5	0
3	0.5	0	5.0	0.15
4	5.0	0.15	0	0.5

Comment: “Day Effect” as a Confounding Factor

- To correct for day effect, days 1 and 2 essentially equivalent and on days 3 and 4 a difference (increase) of 5.5 blinks was observed. To compensate, add 5.5 blinks to all day 1 and 2 data, regardless of dose to “level the playing field” and eliminate day effect.
- No statistically significant increase in eye blink rate due to caprolactam exposure at any time point during exposure, except at 3 hrs using the semi-automated counting method under neon light

OEHHA Response to "Day Effect" as a Confounding Factor

- **"Day effect" statistical analysis relies on:**

  Subjects #1-4 exposed during week 1

  Subjects #5-8 exposed during week 2, etc.

  It is not clear from the Ziegler et al. methodology section that this is the case

- **OEHHA observed a decreasing, not increasing, eye blink trend during the 6 hour exposures**

  Occurred at all control and caprolactam exposures except for the high dose (5 mg/m^3)

  If "day effect" exists, this data suggests a decreasing eye blink trend is more plausible as subject becomes acclimated

OEHHA Response to "Day Effect"

Decreasing eye blink trend during 6 hour exposures

	Eye Blink Analysis	
Caprolactam Concentration	**Statistical Result**	**OEHHA Finding**
0 mg/m^3	p=0.02	Significant decreasing blinks over time
0.15 mg/m^3	p=0.06	Near significant decreasing blinks over time
0.50 mg/m^3	p=0.004	Significant decreasing blinks over time
5.0 mg/m^3	p=0.94	No trend observed

OEHHA Response to "Day Effect" as a Confounding Factor

Blink rate trend with caprolactam exposure dose level is more pronounced than the "day effect" trend for combined time points:

One hour time point, "lights off" method

Page's chi square for dose trend = 9.36 [p=0.002]

Page's chi square for day trend = 1.944 [p=0.16]

Combined time points, "lights on" method

Page's chi square for dose trend = 5.22 [p=0.022]

Page's chi square for day trend = 4.37 [p=0.037]

OEHHA Response to "Day Effect" as a Confounding Factor

- **OEHHA has not encountered any evidence or discussion of a "day effect" by other researchers using similar study protocols**

OEHHA Response to "Day Effect" as a Confounding Factor

In summary:

- The hypothesized confounding by experimental day of exposure is not consistent with this study data.
- There is no precedent from other published studies supporting the proposed reanalysis.
- The rationale for such an effect is not convincing.

Comment: Eye Irritation Confounded by Odor

The subjective eye irritation variable is confounded by odor. The overall odor and eye irritation responses in both the mid and high dose caprolactam groups show a significant ($p<0.05$) correlation by the Spearman test

Response: OEHHA concurs some component of the statistically significant eye irritation trend ($p=0.025$) may be due to odor. This is one reason why the acute REL is based on eye blink rate.

- No effect seen on nasal irritation although odor was recognized: supports eye irritation as a real effect

Comment: No Correlation Between Eye Blink and Eye Irritation

Blink frequency and eye irritation were not correlated in the Ziegler study, contrary to what would be expected if these are “real” caprolactam effects produced by irritation

Response: Haseman’s application of the Spearman correlation test does not take the high individual eye blink variability into account. Also occurs with eye irritation, but less so.

Comment: No Correlation Between Eye Blink and Eye Irritation

Response contd: To account for the variability, we ran a Spearman test of relative eye blink increase vs. absolute eye irritation increase at 1 hour (no-lights) and found a correlation at p=0.01

Applying the same procedure for odor, we also examined relative eye blink vs. absolute odor change at 1 hr:

Spearman's rho = 0.20, so no correlation was found between odor and eye blink rate

Comment: No Eye Redness Observed

There is clearly no caprolactam effect on eye redness, as would be expected if the blink frequency and eye irritation effects are “real” and due to irritation.

Response: Eye redness is an inflammatory response, while increased eye blink frequency is an irritant response that may or may not include an inflammatory component.

Other studies have shown an inconsistent response between these two measures with known irritants, such as formaldehyde.

Comment: Increased Blink Rate Not Biologically Important

Commenter does not view the high dose caprolactam "effect" on overall blink frequency (an increase of less than 9 blinks per 90 seconds) or on mean eye irritation (a mean response not even half way to "barely") as being biologically important responses

Response: Other studies found statistically significant increased eye blink rate in the same range with known irritants. Eye irritation trend not a strong response, which is why REL not based on this endpoint.

Comments on 8-Hr/Chronic REL

- The incidence of labored breathing in animals outside the chamber was very low, sporadic, and did not reflect a dose response. Labored breathing does not constitute organ dysfunction or an adverse effect.

- Secretory observations including red facial stains and clear nasal discharge are common findings in whole-body inhalation studies. Staining and discharge do not represent adverse function of the respiratory tract and cannot be considered as adverse findings.

13-Week Rat Exposure: In-Chamber & Physical Exam Results

	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

- **Red facial stains POD (BMCL05): 4.3 mg/m^3**
- **Clear nasal discharge POD (BMCL05): 6.2 mg/m^3**
- **General condition POD (BMCL05): 3.2 mg/m^3**

Comment: Reinhold Rat Study Vapor Component

The original industrial report states there was an unquantified vapor component to the exposure. If the caprolactam atmosphere presented to the study rats was at a saturation level (13 mg/m^3), then the actual caprolactam exposures were 37, 83, and 256 mg/m^3, not 24, 70 and 243 mg/m^3.

Response: In the study, caprolactam dissolved 1:1 in water and aerosolized. Henry's partition coefficient very small (5.4 X 10^{-11} atm x m^3/mole) suggests vapor component is much smaller than the commenter states.