Comments on the Vinylidine Chloride NTP Bioassay

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On behalf of the VDC producers

Charge Question 2

- Determine whether the study's experimental design, conduct, and findings support the NTP's conclusions regarding the carcinogenic activity and toxicity of the substance tested

NTP conclusions:

Male rats, male and female mice: *clear evidence of carcinogenic activity*

Female rats: *some evidence of carcinogenic activity*

Did the study's experimental design and conduct support these conclusions?

No

2 Key Reasons:

1) Both mice and rat studies **significantly** exceed MTD (minimally toxic dose) according to NTP and EPA guidance
2) Inadequate dose spacing and lack of dose providing a NOAEL

Study is therefore **Inadequate** to assess carcinogenicity hazard and risk to humans, particularly considering the **11** previous bioassays

Rat study MTD

- Dose selection for Bioassay - NTP report states:
  - *Based on the overall minimal chemical-related toxicity in the 3-month study, vinylidene chloride exposure concentrations selected for the 2-year inhalation study in rats were 25, 50, and 100 ppm.*
- All exposed rats in the 13-week study demonstrated clear signs of focal necrosis at dose levels of >12.5ppm
- EPA guidance [(1)]:
  - *Single cell/focal necrosis, if observed at the same dose level in 5-10% of the animals in multiple prechronic/chronic studies, is sufficient evidence that a dose is adequate*
- NTP dose selection strategy (Bucher et al 1996, Fundamental and Applied Toxicology 31, 1-8)
  - *Inhalation studies: Unacceptable lesions included necrosis - MTD should not lead to Unacceptable lesions*
- **12.5ppm** should have been selected as **MTD**

(1) Environmental Protection Agency Health Effects Division (HED) Interim Guidance Document G2003.02, Rodent Carcinogenicity Studies: Dose Selection and Evaluation (July 1, 2003).

MTD in mice

- Dose selection for Bioassay - NTP report states:
  - *Based on mortality, significant reductions in final body weights, and the increased incidences of various nonneoplastic lesions in the 3-month study, vinylidene chloride exposure concentrations selected for the 2-year inhalation study in mice were 6.25, 12.5, and 25 ppm.*
- EPA guidance document [1]
  - MTD is defined as equal to a **depression of body weight gain of 10%**

(1) Environmental Protection Agency Health Effects Division (HED) Interim Guidance Document G2003.02, Rodent Carcinogenicity Studies: Dose Selection and Evaluation (July 1, 2003).

MTD in mice

TABLE 17
Survival and Body Weights of Mice in the 3-Month Inhalation Study of Vinylidene Chloride[a]

Concentration (ppm)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	23.2 ± 0.4	39.4 ± 1.2	16.2 ± 1.1	
6.25	10/10	23.4 ± 0.3	37.8 ± 0.5	14.3 ± 0.3	96
12.5	10/10	23.2 ± 0.2	35.5 ± 0.6**	12.3 ± 0.6**	90
25	10/10	23.4 ± 0.2	33.5 ± 0.8**	10.1 ± 0.8**	85
50	8/10[c]	22.9 ± 0.2	33.0 ± 0.5**	10.0 ± 0.4**	84
Female					
0	10/10	19.6 ± 0.2	35.2 ± 1.2	15.6 ± 1.2	
6.25	10/10	19.5 ± 0.4	30.8 ± 0.6**	11.4 ± 0.7**	88
12.5	10/10	20.1 ± 0.3	31.9 ± 0.9**	11.8 ± 0.7**	91
25	10/10	19.8 ± 0.3	30.9 ± 0.8**	11.1 ± 0.6**	88
50	10/10	19.6 ± 0.4	28.7 ± 0.6**	9.2 ± 0.6**	82
100	6/10[c]	19.5 ± 0.4	29.9 ± 0.8**	10.0 ± 0.4**	85

6.25 ppm dose in the female mouse 90 day study resulted in a bwg depression of 27%, and was 39% in females at 25 ppm - the low and high doses selected for NTP bioassay

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MTD in mice

- Based on BWG and BW, MTD exceeded
- BWG reduction in Chronic bioassay mirrored that in 13 week assay
- MTD should have been 6.25 ppm

Dose Spacing

- EPA guidance [(1)]:
  - *The middle and lowest doses should be selected to **characterize the shape of the dose-response curve** as much as possible. It is important that the doses be **adequately spaced** so that the study can provide relevant dose-response data for **assessing human hazard and risk**. If the testing of potential carcinogenicity is being combined with an evaluation of noncancer chronic toxicity, the study should be designed to include **one dose** in addition to the control(s) that is **not expected to elicit adverse effects**.*
- A factor of 4 separates low and high dose in both studies
- Small dose separation implies greater precision in the assays ability to determine a dose response
- Factor of 10 or greater separation is more ideal
- Tight Dose spacing and Exceeding MTD =
  - No 'No effect level'

(1) EPA Guidelines for Carcinogen Risk Assessment, EPA/630/P-03/001F, March 2005)

Conclusion

- Although findings of the study are interesting
  - Not consistent with previous bioassays (even those performed by NTP)
- Exceeding MTD + inadequate dose spacing – results must be interpreted with extreme caution
- Peer review panel should consider study as ***'Inadequate to assess carcinogenicity to humans'***