

# **Naphthalene Genotoxicity**

## **“Database Assessment”**

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# ***Background***

## **Question**

Does the genetic toxicity profile  
of Naphthalene indicate  
a critical role for  
the compound or its metabolites in its  
carcinogenic effects in rodents?

# ***Background***

- Naphthalene, alone, was tested in 45 studies including *in vitro* and *in vivo* methods. Nine of 45 tests (20%) reported finding some positive effects.
- Naphthalene metabolites (1- & 2-naphthol; naphthalene-1,2-oxide; 1,2- and 1,4-naphthoquinone) were tested directly in 8 studies. Three of 8 (38%) reported finding some positive effects.
- The majority of all studies failed to show any genotoxicity.

# ***Background***

## **Concerns**

1. Responses for a given compound\* in the same genetic endpoint, at comparable dose levels, were conflicting in several of the tests.
2. Some of the responses were not reproducible in subsequent repeat tests by the same testing facility.
3. Some of the endpoints selected for assessment are susceptible to induction by toxic effects as well as direct DNA damage (e.g., DNA fragmentation).

\* Parent or metabolite

# ***Background***

Attempts to interpret the entire Naphthalene genetic toxicology database have concluded that the weight-of-evidence indicates that Naphthalene is not a genotoxic carcinogen.

IARC Monographs, Volume 82, Naphthalene, pp.367-435, 2002.

Schreiner, CA. 2003. Journal of Toxicology and Environmental Health, Part B, 6:161-183.

Butterworth, BE. 2004. Naphthalene Coalition, Expert Opinion.

# ***Issue for risk assessment***

**Should Naphthalene be interpreted as:**

- **Non-genotoxic**
- **Genotoxic with only a secondary or indirect role in the production of rodent tumors.**
- **A genotoxic carcinogen**

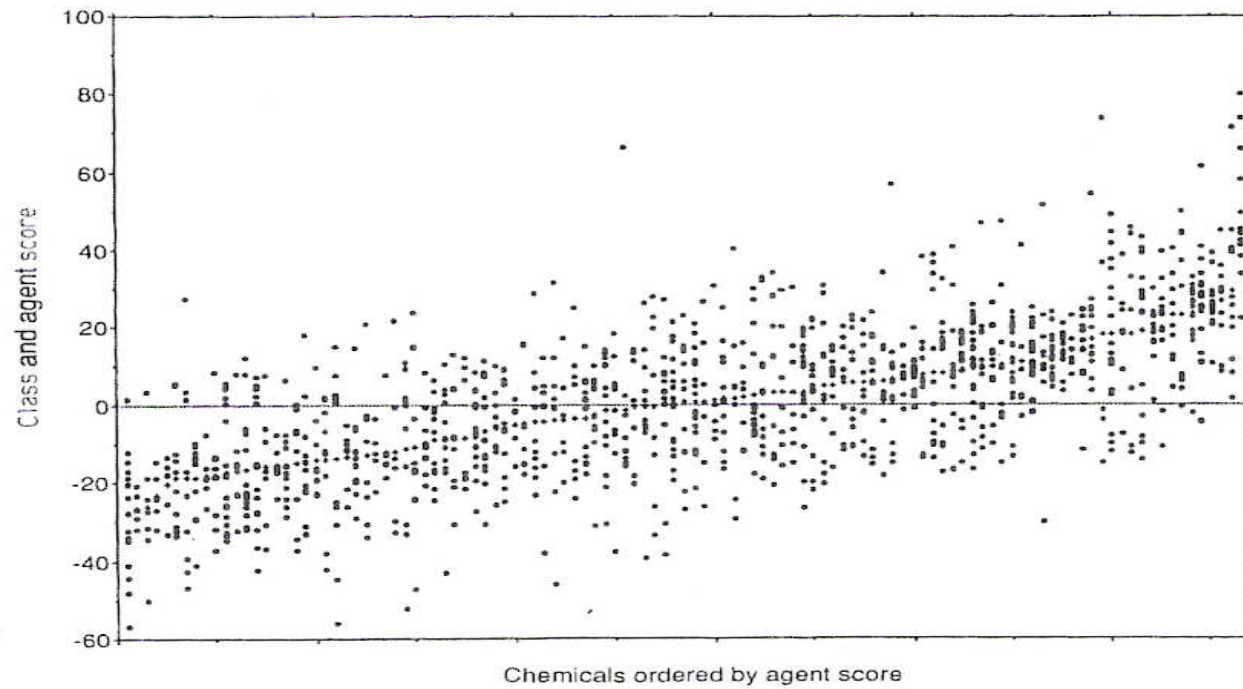
# ***Assessment***

## **How does one determine non-genotoxicity?**

- Difficult to prove a negative
- Intrinsic variability among test methods
- Weight-of-evidence – how much weight?
- Conflicts between *in vitro* and *in vivo* tests
- Are all chemicals positive if tested enough?

# Assessment

Moore et al., 1992. *Mutation Research*, 266: 27- 42



# ***Assessment***

## **Some types of studies that were negative:\***

- Gene (or point mutation) tests in bacteria, cultured mammalian cells with and without S9 mix (17 studies).
- Cell transformation tests *in vitro* (5 studies).
- Chromosome breakage *in vivo* (2 studies).
- Unscheduled DNA synthesis induction in rat livers either *in vitro* or *in vivo* (4 studies).

\* All studies were with naphthalene.

# Assessment

## What appeared to be positive (Naphthalene) ?

- Arfsten et al. 1994. Biomed Environ Sci 7:144-149 (reversion of bioluminescent bacteria “the Mutatox test”).
- Delgado-Rodriguez et al. 1995. Mutat Res 341:235-247 (drosophila wing spot test).
- Djomo et al. 1995. Mutagenesis 10: 223-226 (micronuclei induction in amphibian larvae)
- Gollahon et al. 1990. Toxicologist 10:247 (abst) (chromosome damage in preimplantation embryos *in vitro*)
- NTP, 1992 Report (chromosome breaks in CHO cells *in vitro* and **possible** induction of SCEs). Wilson et al., 1995 found not induction of SCEs using PHLs and H-S9.
- Sasaki et al. 1997. Mutat Res 393: 23-35 (induction of micronuclei *in vitro* in human lymphoblasts)
- Bagchi et al. 2002. Toxicology 175: 73-82 (DNA fragmentation in mouse liver/brain tissue following acute or chronic exposure to Naphthalene)

# ***Assessment***

## **Which positives do not appear to be legitimate concerns?**

- Reversion of bioluminescent bacteria – studies comparing results with the Ames test suggest that any positives should be confirmed using the standard Ames test (Jarvis et al. 1996. *Ecotoxicol Environ Saf*, 33: 193-200). 15 standard Ames tests with Naphthalene are all negative.
- *Drosophila* wing spot test – in addition to naphthalene, anthracene and phenanthrene, both non-carcinogens, were positive in this assay.
- Induction of micronuclei in salamander larvae – experimental treatment conditions and unknown metabolic detoxification processes and lack of historical data make extrapolation of this data to mammalian hazard impossible.

# ***Assessment***

## **The remaining positive studies indicate that:**

- Naphthalene, or more likely, one (or more) of its metabolites is genotoxic.
- The genetic effects reported for Naphthalene are consistent with metabolite-induced DNA breakage events leading to chromosome aberrations, micronuclei or SCE, but not to gene mutation.
- The DNA fragmentation events produced *in vivo* do not appear as micronuclei or UDS. The effects observed may be explained by release of lysosomal enzymes linked to toxicity that would degrade DNA during sample preparation.

# ***Assessment***

## **Naphthalene metabolites**

1,2-naphthoquinone and 1,4-naphthoquinone exhibit some evidence for genotoxicity in several genetic tests, but the epoxide and 1-naphthol metabolites did not.

### **Types of genotoxic effects reported:**

- Chromosome breakage (micronuclei in human lymphoblastoid cell line MCL-5)
- SCE in human lymphocyte cultures
- Reversion of *S. typhimurium* strains TA100 and TA104

# ***Assessment***

Napthoquinones:

1. Highly reactive producing cytotoxicity through oxidative damage.
2. Genotoxic effects reduced or eliminated by free radical scavengers and S9 mix.
3. Genotoxic effects highly correlated with cytoxic damage.

# ***Assessment***

## **Naphthalene is not effective in producing gene mutation**

- ~15 negative Ames reports with TA100 and S9 mix.
- Sakai et al., 1985 failed to show positive effects for 1,4 naphthoquinone in the standard strains including TA100.
- Hakura et al., 1994 and Flowers-Geary et al., 1996 reported positive responses in TA104 and TA100 (responsive to oxidative damage) for both quinone metabolites in the absence of S9 mix (addition of S9 significantly reduced the positive effects).
- The Hakura studies demonstrated that the positive effects were at concentrations equivalent to 90% killing of the target cells.
- Sasaki et al., 1997 failed to induce gene mutation with 1,4-naphthoquinone in human cells at the tk locus.

# ***Assessment***

## **Naphthalene metabolites produce chromosome breakage *in vitro***

The effects are produced at concentrations exceeding the intrinsic capacity for detoxification and are therefore cytotoxic for the target cells.

- The effects are consistent with a mechanism linked to DNA breaks induced by cytotoxic oxidative damage and not direct DNA binding.
- At doses that are not excessively toxic, animals are able to detoxify the reactive species effectively as demonstrated by the negative findings in all *in vivo* studies reported.

# ***Conclusions***

- Large heterogeneous database indicating that Naphthalene has a limited ability to damage DNA and produce stable lesions.
- DNA breakage induced by reactive oxidation species found only at cytotoxic levels.
- No evidence that this mechanism is relevant *in vivo*.

# ***Conclusions***

## **Modes of Action**

***Driving***: responsible for the conditions establishing the neoplastic process at the target site(s).

***Contributory***: secondarily facilitates the neoplastic process once specific conditions are active at the target site(s).

# ***Conclusions***

1. A set of events consisting of target site cytotoxicity, inflammation and induction of cellular regeneration appears to be the driving mode of action for Naphthalene.
2. Naphthalene genetic toxicology data are consistent with a contributory mode of action in which chromosomal alterations may be part of the overall process.