

Naphthalene Genotoxicity

“Database Assessment”

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Background

Question

Does the *genetic toxicity* profile
of Naphthalene indicate
a primary role for the chemical in rodent
carcinogenicity?

Background

Prior assessments of the Naphthalene genetic toxicology database concluded that the weight-of-evidence indicated that Naphthalene does not act through a genotoxic mode of action. However, there were still concerns.

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.

Background

- The majority of all studies failed to show any genotoxicity
- 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.*

Background

Concerns about the database

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 positive 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

***The Issue relevant to
Naphthalene risk assessment***

**Should Naphthalene be
classified as:**

- Non-genotoxic
- A genotoxic carcinogen
- Genotoxic with possible secondary or contributory role in the production of rodent tumors (favored by Schreiner)

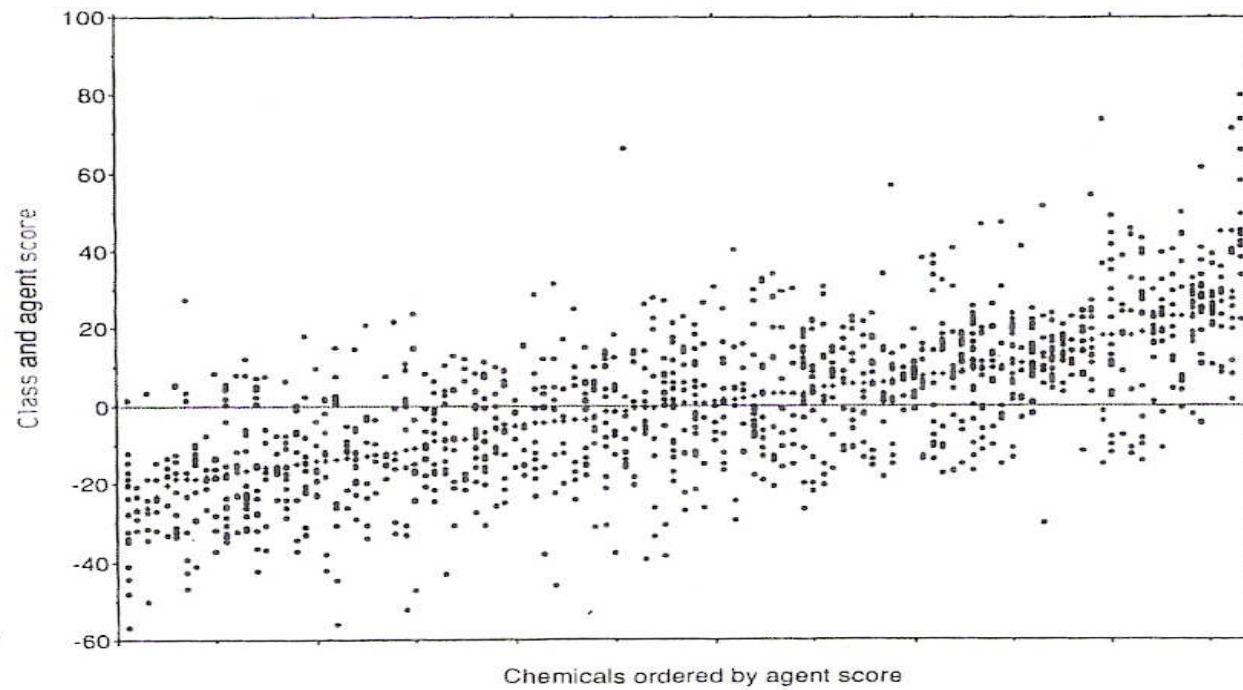
Assessment

How does one determine non-genotoxicity?

- Difficult to prove a negative
- Intrinsic variability among test methods
- Weight-of-evidence – how much is sufficient?
- 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

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).
- Bacterial tests for induction of repairable DNA damage.

* All studies were with naphthalene.

Assessment

What appeared to be positive (Naphthalene) ?

(could focus on the positive studies reported)

- 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* with the addition of S9).
- NTP, 1992 Report (chromosome breaks in CHO cells *in vitro* and **possible** induction of SCEs). Wilson et al., 1995 found no 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:

- *In vitro* positive responses with Naphthalene required or were amplified with addition of S9, making it likely that one (or more) of its metabolites is genotoxic under specific conditions.
- The genetic damage reported for Naphthalene is most consistent with metabolite-induced DNA breakage events leading to chromosome aberrations, micronuclei or SCE, but not *generally* to gene mutation.
- The DNA fragmentation events produced *in vivo* do not appear as micronuclei or UDS in separate studies. The breakage 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, but the epoxide and 1-naphthol metabolites consistently did not.

Types of genotoxic effects reported:**

- Chromosome breakage *in vitro* (including micronuclei in human lymphoblastoid cell line MCL-5)
- SCE in human lymphocyte cultures.
- Reversion of *S. typhimurium* strains TA100* and TA104* in the absence of S9. S9 addition eliminated the response.

* Susceptible to oxidative damage

** None were obviously flawed

Assessment

Napthoquinones and chromosome effects:

1. Highly reactive producing cytotoxicity through oxidative damage to DNA and other macromolecules likely producing abasic sites and single strand breaks.
2. Genotoxic effects reduced or eliminated by free radical scavengers and S9 mix.
3. Genotoxic effects highly correlated with cytotoxic damage.

Assessment

Naphthalene was not very effective in producing gene mutation

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

Assessment

Naphthalene metabolites produce predominantly chromosome breakage *in vitro*

The effects are produced at concentrations probably exceeding the intrinsic cellular 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 damage. Evidence for direct DNA binding is very limited.
- At doses that are not excessively toxic, animals appear able to detoxify the reactive species effectively as demonstrated by the negative findings for chromosome breakage or UDS in all *in vivo* studies reported.

Conclusions

- Large heterogeneous database suggesting that Naphthalene, **alone**, has limited ability to damage DNA or produce stable lesions leading to base changes.
- DNA breakage induced by highly reactive metabolites found under cytotoxic treatment conditions leads to chromosome alterations *in vitro*.
- No evidence in the database that this breakage mechanism leads to chromosome aberrations *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 generated at the active target site(s).

Conclusions

1. A set of events consisting of target site toxicity and the 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 (possible base substitutions) may be part of the overall process in the target cells.

Additional Data

Information provided at this meeting indicating that specific depurinating adducts produced by hydrocarbon/estrogen metabolites can lead to base substitution mutations in tumor initiating genes.

Working Assumption

- *Depurinating adducts produced at sites that are not associated with tumors.*
- *Suggests that other variable(s) must drive the tumor induction process.*

Research Opportunity

Determine how the tumor sensitive and resistant sites differ.

For example:

- *Appropriate activating enzymes*
- *Differential DNA repair*
- *Cell turnover/proliferation*
- *Inflammation*
- *Other*