
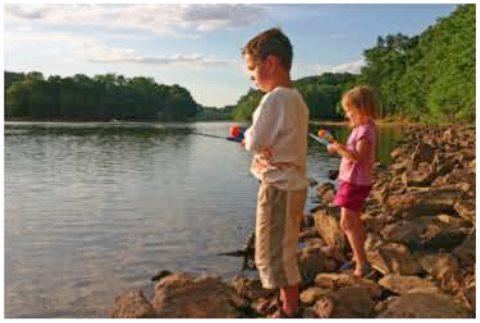

Science Question 4: Comments on the USEPA Mechanistic Studies Database

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Overview of Comments

Currently, the information in the database as well as associated summary figures/tables in the preliminary materials are inaccurate:

- **Comment 1**: The database is missing critical studies
- **Comment 2:** An inconsistent approach was used to classify entries in the database as “mutation” outcomes
- **Comment 3**: The database contains a number of inconsistent and inaccurate entries for the mutagenicity outcomes
- **Comment 4:** The accuracy of the entire database was called into question based on an independent review of mutation outcomes; a systematic approach is required prior to further assessment

Comment 1 – Key Studies Missing from Database

Four key studies with mechanistic data were identified as missing from the database:

1. Thompson et al. (2011). **Investigation of the mode of action underlying the tumorigenic response induced in B6C3F1 mice exposed orally to hexavalent chromium.** *Toxicological Sciences* 123, 58-70.
2. Thompson et al. (2012a). **Comparison of the effects of hexavalent chromium in the alimentary canal of F344 rats and B6C3F1 mice following exposure in drinking water: implications for carcinogenic modes of action.** *Toxicological Sciences* 125, 79-90.
3. Thompson et al. (2012b). **Assessment of Cr(VI)-Induced Cytotoxicity and Genotoxicity Using High Content Analysis.** *PLoS One* 7, e42720.
4. Suh et al (2014). **High concentrations of hexavalent chromium in drinking water alters iron homeostasis in F344 rats and B6C3F1 mice**. *Food Chemical Toxicology* 65, 381-388.

Comment 2 – Inconsistent and Erroneous Classification of "Mutation" Outcomes

- Repetition and inconsistencies with outcome labeling were found in the mutation entries (yellow)
  - i.e. all refer to the same type of assay outcome
- Inconsistent outcome category assignment (blue)
  - Approximately half of the micronuclei and chromosomal aberration outcomes were placed in the mutation category and the other half in DNA damage category
- Several entries appear to be classified as mutation outcomes in error (red)
- Misclassified mutation endpoints (blue)
  - Chromosome aberrations
  - Micronuclei

Outcomes	# entries
1-NP nitroreductase activity	1
8-azaguanine resistant colony formation	2
Aberrant colonies	2
abnormal metaphase	2
abnormal metaphase cells	1
appearance	1
Ar reversion	3
Ara mutants	1
CA	4
Cell survival	1
cell viability	1
centromere spreading	1
characterization of revertant colony genotype	1
Chromasomal mutation	1
Chromate reduction	1
chromatid-type aberrations	1
chromosomal aberration	2
chromosomal aberrations	20
chromosomal mutation	11
chromosome aberration	5
chromosome aberration frequency	7
chromosome breaks	3
chromosome damage	12
chromosome damage and repair	1
chromosome damage/instability	1
chromosome instability	1
chromosome-type aberrations	1
colony formation	3
complexation and chelating	1
cytoplasmic bridges	1
DCF fluorescence	1
development of a new cell culture model	1
DNA damage	2
DNA double-strand breaks	1
flocculation	1
Forward mutation	1
frequency of micronuclei	1
Gene convertants	2
Gene mutation	20
gene mutations	2
Gene revertants	2
HGPRT	2
HIS reversion	5
hprt mutation frequency	3
HPRT mutations	1
incorporation of 1-NP into cells	1
indirect immune-rosetting reaction (iIRR)	1
K-Ras codon 12 GAT mutation	1
lacZ- mutant plaques	4
Lys^+ reveratants	1

Continued next column.

Outcomes, continued	# entries
mammalian spot test	2
micronucleated cells	1
micronuclei induction	5
micronucleus	8
micronucleus frequency	2
micronucleus induction	1
mitochondrial cytochromes	1
mitotic gene conversion	1
MN induction	1
mutagenic activity	16
mutagenic effects	5
mutagenic frequency	1
mutagenicity	13
mutant frequency	5
Mutation	7
mutation frequency	24
mutation frequency at HPRT locus	1
mutation identification	2
mutation in bacterial lacI gene in bacteriophage shuttle vector	2
mutation in shuttle vector plasmid YCpMP2	1
mutation in shuttle vector pZ189	1
mutation of lacZ gene	2
Mutation sequence	2
mutation spectrum	5
mutations	6
number of chromosomes	1
number of mutations	1
other nuclear anomalies	1
petite frequencies	1
Plasmid survival	1
polyploid cells	1
postimplantation embryo loss	1
premature anaphase	1
premature centromere division	1
reduction	1
Reduction of Cr(VI) to Cr(III) by gastric juice, by saliva and by erythrocyte lysates; deactivation of Cr(VI) mutagenicity by S9 fractions from various tissues	1
Reverse mutation	1
reverse mutation induced by 1-NP	2
reversion mutagenesis	1
reversion mutations	1
revertants	28
Site specific oxidation patterns	3
Transformation of E.coli by HEK293-replicated plasmids	1
X-gal mutation	2

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Comment 3 – Inconsistent and Inaccurate Entries within the Mutagenicity Outcomes

- The 311 entries characterizing mutagenicity do not all represent outcomes associated with hexavalent chromium.
  - A number of entries represent a chromium oxidation state other than hexavalent
- The separation of studies (144) into entries (311 rows) by chromium compound/valence state was inconsistent.
  - Some entry rows combined up to 12 compounds and several valences into one
- The separation of studies (144) into entries (311 rows) by cell type/strain was inconsistent.
  - Some entry rows combined multiple cell lines or strains while others listed them as separate outcomes
- The USEPA did not separate entries by route of administration.
  - At least three instances (DeFlora et al., 2006; Mirsalis et al., 1996; Newton and Lilly, 1986) where several routes of administration were combined into one outcome
- The USEPA database contains duplicate and repetitive entries.
  - Inclusion of unoriginal data (Patierno and Landolph, 1989) and incorrect/duplicated citations (De Flora et al. 1984 and DeFlora et al. 1985) resulting in inflated counts