Guideline Studies
Chronic Toxicity
Carcinogenicity

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Workshop
Threshold of Toxicological Concern (TTC)
for risk assessment of food contact material chemicals
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Threshold of Toxicological Concern Databases

The presently used TTC values are derived from two databases reporting effects after oral exposure*

1- Carcinogen Potency Database (730 chemicals)
   -NCI/NTP Carcinogenesis Bioassay Program (up to July 1980)
   -Bioassays in the published literature from 1948 - 1981

2- Munro Analysis (613 chemicals)
   -grouped chemicals according to decision tree of Cramer *et al.*[&]

   -1921-1993, 44% studies before 1981

   -industrial chemicals, pharmaceuticals, food substances, environmental, agricultural and consumer chemicals

*http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_092.pdf

1-Gold *et al. Env Health Perspect*, 58: 9-319, 1984 2-Munro *et al. Food Chem Toxicol* 34:829-867, 1996
& Cramer *et al. Fd Cosmet Toxicol* 16:255-276, 1978

Guideline Development

- **US Food and Drug Administration** first guidance to industry for assessing toxicity of chemicals in food **1949**
  - Three doses: maximal tolerated dose, intermediate, 100 times the amount proposed to use in food

- **WHO** Procedures for testing of international food additives to establish their safety for use in **1958**
  - Two dose levels plus one control group

1950's marked the first attempts at standardized testing with an emphasis on qualitative hazard identification with a focus on food additives and pharmaceuticals

Jacobs and Hatfield Vet Path 50: 324-333, 2012, Beyer *et al. Crit Rev Toxicol* 41:321-338, 2011
OECD Draft Guidance Document Nº 116 http://www.oecd.org/chemicalsafety/testing/44076587.pdf

Guideline Development

- **1960s/1970s**
  - **1970's** increasing reliance on bioassays for assessment of occupational and environmental hazards
  - degree of standardization for bioassays sought
    - Deficient in details regarding dosing, length of treatment, no or imperfect controls

- **National Cancer Institute** Guidelines for Carcinogen Bioassay in Small Rodents **1976**

- **1970s/1980s** use of formal risk assessment procedures began with interest in using data from animal studies for <u>quantitative purposes</u>
  - 1973 FDA proposed procedure for calculating maximum acceptable carcinogen intake
  - 1976 EPA established guidelines for assessing risk from chemicals in the environment

Beyer *et al. Crit Rev Toxicol* 41:321-338, 2011
OECD Draft Guidance Document Nº 116 http://www.oecd.org/chemicalsafety/testing/44076587.pdf

Guideline Development

- **Organization of Economic Cooperation and Development (OECD)** Test Guidelines **1981**
  - TG 452 Chronic Toxicity **(2009 update)**
  - TG 451 Carcinogenicity (updated draft)
  - TG 453 Combined (updated draft)

- **US Food and Drug Administration** Guidelines**:** Redbook 1 **1982** updated to Redbook **2000**
  - Chapter IV.C.5.a. Chronic Toxicity,
  - Chapter IV.C.6 Carcinogenicity,
  - Chapter IV.C.7 Combined

- **US Environmental Protection Agency Guidelines: 1986** updated **1998**
  - OPPTS 870.4100 Chronic
  - OPPTS 870.4200 Carcinogenicity
  - OPPTS 870.4300 Combined

OECD Draft Guidance Document Nº 116 http://www.oecd.org/chemicalsafety/testing/44076587.pdf

Guidelines for Carcinogen Bioassay in Rodents Chronic Study (1976)

Objective

-“ to determine the carcinogenicity of the test agent in both sexes of two species and is designed to cover the greater part of the animals´ life span”

National Cancer Institute Carcinogenesis Technical Report Series No. 1 February 1S76, 1976
http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr001.pdf

Guidelines for Carcinogen Bioassay in Rodents Chronic Study (1976)

Exposure

-animals not older than 6 weeks of age, possible weanlings (50 per group)
-given for a sufficient time to produce a maximum response (usually 24 months)

Dose Levels

-two dose levels
    -Highest dose: maximum tolerated dose
    -Lower dose: a fraction thereof (typically 50 or 25%)

National Cancer Institute Carcinogenesis Technical Report Series No. 1 February 1S76, 1976
http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr001.pdf

Guidelines for Carcinogen Bioassay in Rodents Chronic Study

Observation

-viability on daily basis

-palpation and exam once weekly

-monthly body weight, feed consumption on a regular basis

-necrospy

-external examination, all organs are to be examined *in situ* and following excision

-peripheral blood smears

-histopathologic examination of all control and treated animals

National Cancer Institute carcinognesis Technical Report Series No. 1 February 1S76, 1976
http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr001.pdf

Chronic Toxicity Studies TG 452
OECD Guidelines 1981

Objective

"characterize the profile of a substance in a mammalian species following prolonged and repeated exposure"

It should generate data to:

- identify the majority of chronic effects
- determine dose-response relationships

OECD Guideline for Testing of Chemicals Adopted 12 May 1981
http://www.oecd.org/env/ehs/testing/45125416.pdf

Chronic Toxicity Studies TG 452

Selection of Species

two mammalian species
well characterized strains

Exposure

to begin after weaning and acclimatisation
duration for 12 months
20 animals (rodent), minimum 4 (non-rodent) / sex / dose level

Dose levels

3 dose levels + vehicle control
Highest: elicit signs of toxicity without excessive lethality
Lowest: should not produce toxicity

OECD Guideline for Testing of Chemicals Adopted 12 May 1981
http://www.oecd.org/env/ehs/testing/45125416.pdf

Chronic Toxicity Studies TG 452

Observations

1- daily clinical exam for signs of toxicity, overt neurological and ocular changes, mortality

2-body weight / week for the first 13 weeks and then every 4 weeks

3-food intake / week for the first 13 weeks and then at 3 month intervals

4-hematology: at 3 and 6 months and termination
-hemoglobin content, PCV, total RBC, total WBC, platelets

5- differential blood counts highest dose and control groups

OECD Guideline for Testing of Chemicals Adopted 12 May 1981
http://www.oecd.org/env/ehs/testing/45125416.pdf

Chronic Toxicity Studies TG 452

Observations

6-urinalysis: volume, protein, glucose, ketones, occult blood, sediment

7- clinical chemistry at 6 months and termination
- total protein, albumin, liver enzymes, glucose, blood urea nitrogen

8-pathology
- gross necropsy/macroscopic
- microscopic examination of preserved organs (40 suggested)
  - -all of the highest dose and control group
  - -prematurely died or euthanized
  - -any organs showing macroscopic abnormalities

OECD Guideline for Testing of Chemicals Adopted 12 May 1981
http://www.oecd.org/env/ehs/testing/45125416.pdf

Points of Consideration

1. **Objectives of the guideline studies/selection of doses**

Emphasis of experimental designs on hazard identification

vs.

Identification of robust thresholds for quantitative risk assessment

Chronic Toxicity Studies TG 452

OECD Guideline Adopted 7 September 2009

Objectives

- identification of hazardous properties of a chemical
- identify target organs
- characterization of the dose : response relationship
- identify NOAEL (no observed adverse effect level) or point of departure for establisment of a Benchmark Dose
- prediction of chronic toxicity effects at human exposure levels
- provision of data to test hypotheses regarding mode of action

http://www.oecd-ilibrary.org/docserver/download/9745201e.pdf?expires=1381776960&id=id&accname=guest&checksum=92613EEB46781A584BEEF52006FF1DC2

Points of consideration
2. Timing of Exposure

Chronic bioassays: “exposure as soon as possible after weaning” (PND 21 in the rat) and no later than 6 to 8 weeks

Inclusion of published literature in the carcinogenicity database stipulated that exposure had to commence before PND 100

In utero and early postnatal development +/- puberty have not been routinely evaluated

2. Timing of Exposure

Development is a period of increased sensitivity to external exposures

- experimental teratology: periods of high sensitivity for producing certain types of malformations
- epidemiological and animal studies disorders of homeostatic systems, such as diabetes and hypertension, which could be affected by programming in early life
- organizational/permanent changes following developmental exposure *vs* activational/reversible changes following exposure in adulthood

Rhomberg *et al. Crit Rev Toxicol*. 37:729-837

2. Timing of Exposure
Carcinogenicity

Quantitative and/or qualitative differences between fetus/neonate *vs.* adult

- Fetal and neonatal metabolism
  - lack of carcinogen detoxification capacity can increase risk for tumor formation
  - lack of activational enzymes could lead to a decreased risk
- Maternal metabolism
  - Increase of risk: enzyme activation of carcinogens to stable intermediates which are active
  - Decrease of risk: detoxification and clearance of the chemical
- Fetus and neonate are more sensitive due to rapid rate of cell division
  - persistence of mutations before repair can occur

Anderson, *Toxicol Appl Pharmacol* 199: 162-74, 2004

Last Thoughts

How robust are NOELs derived from data obtained from experimental designs emphasizing hazard identification?

How do we assess risk for developmental exposures when adequate data for this is lacking in the databases used to derive the TTC values?

Comparison NOEL

Substance	NOEL Munro *et al. Food Chem Toxicol* , 1996	NOAEL /LOAEL used for opinion	Source
Bisphenol A	56 mg/kg/d Body weight (1982)	5 mg/kg bw/d NOAEL Liver Toxicity	European Food Safety Authority §
Dibutyl phthalate	125 mg/kg/d Lethality (1953)	2 mg/kg bw/d LOAEL germ cell development/ mammary gland	European Food Safety Authority $
Bis (2-ethylhexyl)phthalate	18 mg/kg/d Reproductive (1986)	5 mg/kg bw/d NOAEL Testicular/developmental toxicity	European Food Safety Authority *
Diethyl phthalate	2218 mg/kg/d Body weight (1955)	150 mg/kg bw/d NOAEL Liver weight	SCCNFP/0411/01 European Commission &
Diethylene glycol	1250 mg/kg/d multiple effects (1991)	50 mg/kg bw/d Cystolithiasis	SCCP/1181/08 Health and Consumer Protection Directorate General European Commission %

§ The EFSA Journal (2006) 428 http://www.efsa.europa.eu/en/efsajournal/doc/428.pdf
$ The EFSA Journal (2005) 242, http://www.efsa.europa.eu/de/efsajournal/doc/242.pdf
* The EFSA Journal (2005) 243, http://www.efsa.europa.eu/en/efsajournal/doc/243.pdf
& http://ec.europa.eu/health/ph_risk/committees/sccp/documents/out168_en.pdf(2002)
% http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_139.pdf(2008)

3. Timing of Exposure

Important periods of neurological development in the rat

GD6-15 formation of neural tube, beginning of blood brain barrier formation

GD15-21 neuronal migration

PND 1-9 brain growth spurt (synaptogenesis)

Most of the developmental neurotoxicity studies did not expose the test animals over the entire critical window of development as recommended by the EPA guidelines (GD6-PND10)

Kroes et al., *Food Chem Toxicol 38*: 255-312, 2000
US EPA Guidelines OPPTS 870.6300, 1996

Points of Consideration

2. Selection of doses in guideline studies

Previous emphasis on maximally tolerated doses followed by the use of one or two more doses

Limits the amount of information at dose levels

-near the range of suspected thresholds for

-specific modes of action or
-metabolism

where robust estimation is needed to perform risk assessment

-relevant for human exposure scenarios