



A Weight of Evidence Approach to Cancer Assessment

Alan R Boobis

Imperial College London

a.boobis@imperial.ac.uk

Disclosure Statement

- Member of Board of Trustees of ILSI, Board of Directors of ILSI Europe and Board of Trustees of HESI
- Involved in several expert groups and technical committees within ILSI Europe and HESI addressing generic risk assessment issues
- Chair of the UK Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT)
- Member of the UK Committee on the Medical Effects of Air Pollutants (COMEAP)
- Member of the FAO/WHO Joint Meeting on Pesticide Residues (JMPR)
- Member of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (Residues of veterinary drugs)
- Member of the Science Advisory Board of the Swiss Centre for Applied Human Toxicology (SCAHT)
- Member of the External Advisory Committee for the Center for Research on Ingredient Safety (CRIS), Michigan State University, USA
- Member of the Scientific Advisory Board of Owlstone Medical
- Member of the Science Advisory Board of Cosmetics Europe Long Range Research Strategy
- Member of the Scientific Advisory Board of Innovations in Food and Chemical Safety Programme, A*STAR, Singapore
- Grant support from the European Commission under Horizon 2010 for research on the cumulative toxicity of chemicals in food (EuroMix)



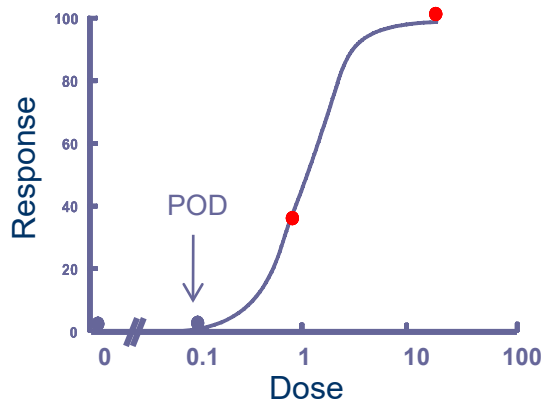
Hazard and Risk Assessment



Hazard ID
Hazard characterisation



Reference value (e.g., ADI)
[RV] = POD/UF



Risk characterisation

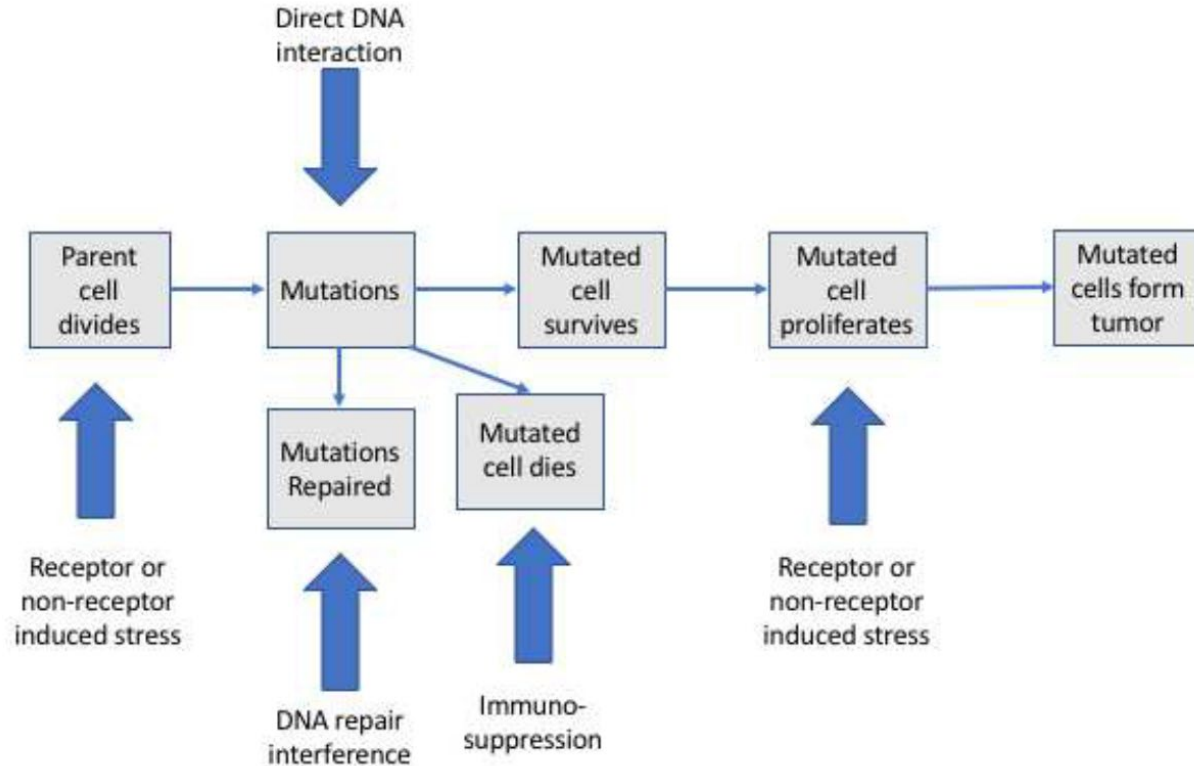


The Cancer Bioassay

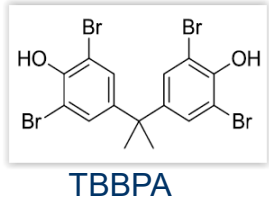
- Compounds are tested in rodents (rats and mice) for a lifetime, at high doses, to facilitate hazard identification
- The basis of hazard identification is tumour incidence
- Many chemicals cause carcinogenicity secondary to their toxicity, i.e., via a non-genotoxic mechanism
- The relevance of tumours produced at high, toxic doses of a chemical to human health risk is highly questionable
- Some mechanisms of carcinogenicity in rodents are of little or no relevance to humans



Chemical Carcinogenicity



3,3',5,5'-Tetrabromobisphenol A (TBBPA)



- CAS No 79-94-7
- MW 543.9
- Log K_{OW} 9.7 (protonized); 3.2-6.4 (neutral pH)
- pK_a 7.5/8.5
- Used primarily as a reactive flame retardant, covalently bound to epoxy and polycarbonate resins
- Also used as an additive flame retardant

Repeat-Dose Toxicity of TBBPA

- 90-day study in rats:
 - Decreased serum bilirubin in males at 1000 mg/kg bw and in females at 300 mg/kg bw
 - Slight changes in T4 at highest dose
- Two-generation study in rats
 - Decreases in T4 at 100 mg/kg bw and 1000 mg/kg bw
- Developmental toxicity study in rats
 - Diffuse thyroid follicular cell hypertrophy in dams at 100 mg/kg bw
- No evidence for reproductive or developmental toxicity

Immunotoxicity of TBBPA

- Some evidence for effects on host immunity in mice at 1700 mg/kg bw per day for 28 days
- No effect on immunisation response to SRBC in rats at up to 3000 mg/kg bw per day for 28 days

Hepatic Effects of TBBPA

- 90-day study in rats
 - Decreased serum bilirubin in males at 1000 mg/kg bw and in females at 300 mg/kg bw
 - Serum alkaline phosphatase levels increased at 1000 mg/kg bw in females
 - No other effects on clinical chemistry and no histopathological changes in the liver
- 28-day study in rats
 - No effect on CYP mRNA levels at doses up to 300 mg/kg bw
- GD0-GD27 treatment of pregnant mice with up to 1% in diet
 - Increased liver weights in dams and offspring
 - Increase in focal necrosis of hepatocytes and inflammatory cell infiltration in the liver of dams and offspring
- 14-day study in male mice
 - Increased liver weights at 1400 mg/kg bw
 - Some evidence of histopathological changes in liver from 350 mg/kg bw

Genotoxicity Studies of TBBPA

- No evidence of genotoxicity in *S. typhimurium* TA92, TA98, TA100, TA1535, TA1537, or TA1538, or in *Saccharomyces cerevisiae* strains D3 or D4, with or without metabolic activation at up to mg/plate
- No evidence for induction of chromosomal aberrations in isolated human lymphocytes at up to 75 µg/ml, with or without metabolic activation
- No evidence for induction of SCEs in Chinese hamster ovary (CHO) cells at up to 500 µg/ml, with or without metabolic activation
- No evidence for induction of unscheduled DNA synthesis in isolated rat hepatocytes, at up to 1,000 µg/ml

Characterization of Carcinogenic Risk of TBBPA

- EFSA CONTAM 2011 (EFSA J 2011;9(12):2477)
- Absence of genotoxicity *in vitro*
- No indications for proliferative changes or cytotoxicity in studies with up to 90 days repeated administration
- No immunosuppression, except possibly at high doses
- The CONTAM Panel concluded that, based on the weight of evidence, there are no indications that TBBPA might be carcinogenic
- The critical effect was a decrease in circulating thyroid hormone (T4) levels in rats. A BMDL10 of 16 mg/kg b.w. per day for effects in females was used to calculate MOEs

NTP Cancer Bioassay of TBBPA (2014)

- 90-day study in F344/NTAC rats confirmed decreased T4 levels from 500 mg/kg bw. Liver weights were increased at these doses, with no changes in hepatic histopathology
- 90-day study in B6C3F1/N mice: Liver weights were increased from 500 mg/kg bw. At these doses, effects were also seen on the kidney
- Two-year study in Wistar Han rats
 - Clear evidence of carcinogenic activity in females, based on increased incidences of uterine epithelial tumours (predominantly uterine adenocarcinoma)
- Two-year study in B6C3F1/N mice
 - Some evidence of carcinogenic activity in males, based on increased incidences of hepatoblastoma

Lesions of Rat Uterus in Two-Year Study of TBPPA

N = 50

Lesion	Control	250 mg/kg bw	500 mg/kg bw	1000 mg/kg bw
Endometrium, Hyperplasia, Atypical	2	13**	11**	13**
Adenoma	3	2	4	6
Adenocarcinoma	4	10	15*	16*
Malignant Mixed Müllerian Tumour	0	4	0	2

NTP, 2014

Critical BMDL10 (EFSA): 16 mg/kg b.w. per day

Lesions of Mouse Liver in Two-Year Study of TBPPA

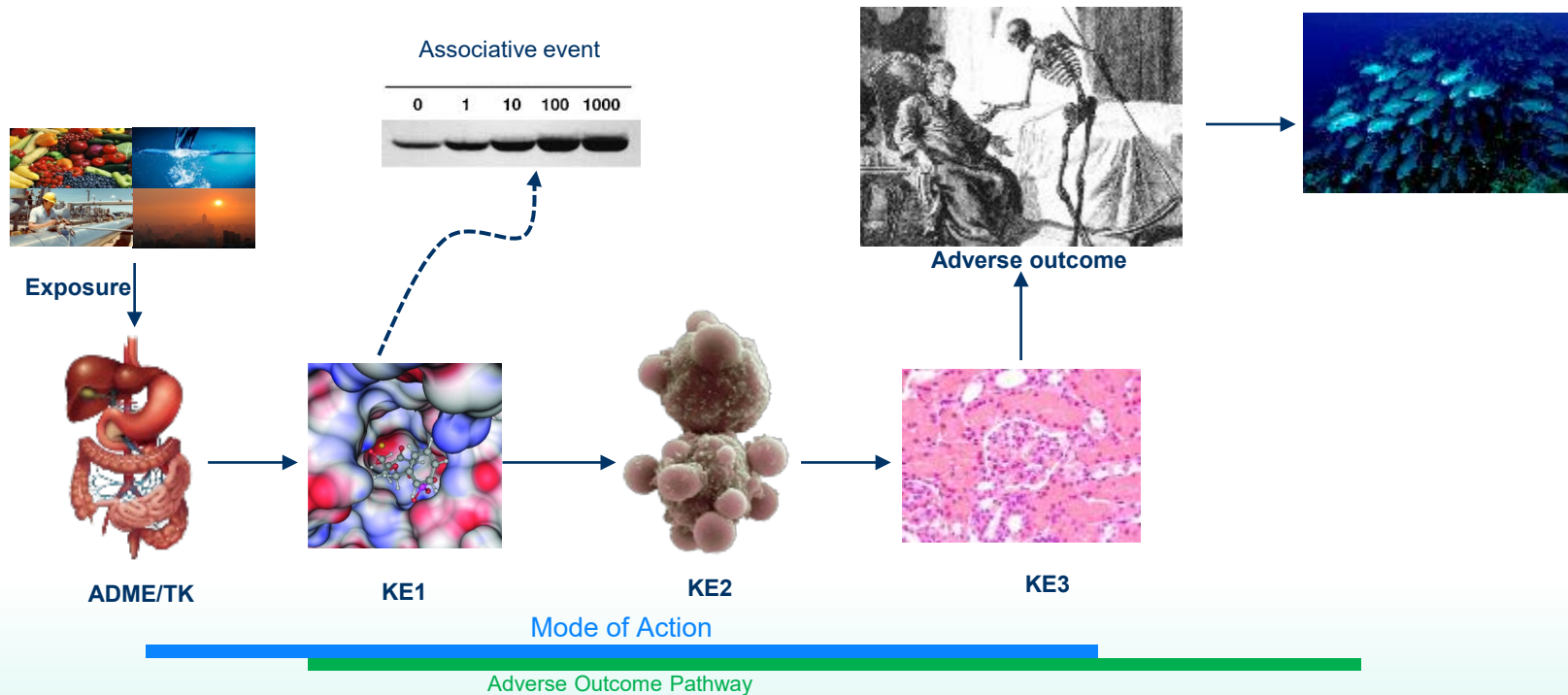
N = 50

Lesion	Control	250 mg/kg bw	500 mg/kg bw
Clear Cell Focus	11	10	25**
Eosinophilic Focus	20	33**	40**
Hepatocellular Adenoma, Multiple	12	20	28**
Hepatocellular Adenoma or Hepatocellular Carcinoma	39	39	43
Hepatoblastoma (Poly-3: P = 0.065)	2	11*	8
All (adeno, carcino, hepatbl'a)	39	42	43

NTP, 2014

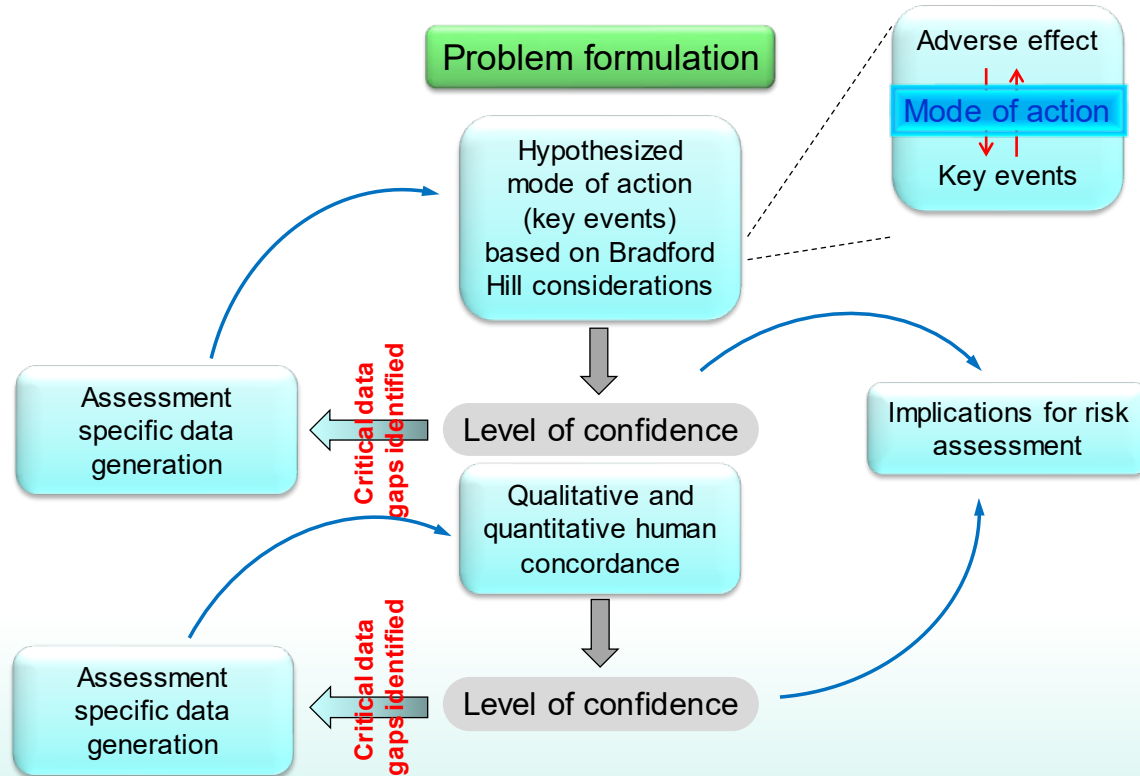
Critical BMDL10 (EFSA): 16 mg/kg b.w. per day

Mode of Action (MOA)/Adverse Outcome Pathway (AOP)

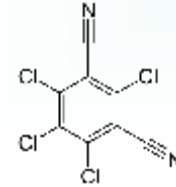


Key events (based on Bradford Hill considerations)

IPCS Human Relevance Framework

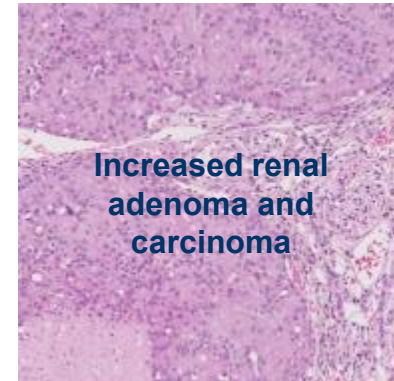


Risk Assessment: MOA for Renal Carcinogenicity



Chlorothalonil

- Classify as a carcinogen?
- Manage risk of renal toxicity?



GSH- and downstream metabolites found in urine

GSH conjugation and further metabolism to Cys conjugates

Accumulation of radiolabel in kidney; inhibition by probenecid

Active uptake of Cys conjugate by proximal convoluted tubule

Evidence from studies with analogous compounds

Metabolism to thiols by C-S lyase

Consistent time- and dose-dependent histopathological and clinical chemical evidence for toxicity

Renal cytotoxicity

Proliferation observed within 7 days; dose and time-dependent hyperplasia

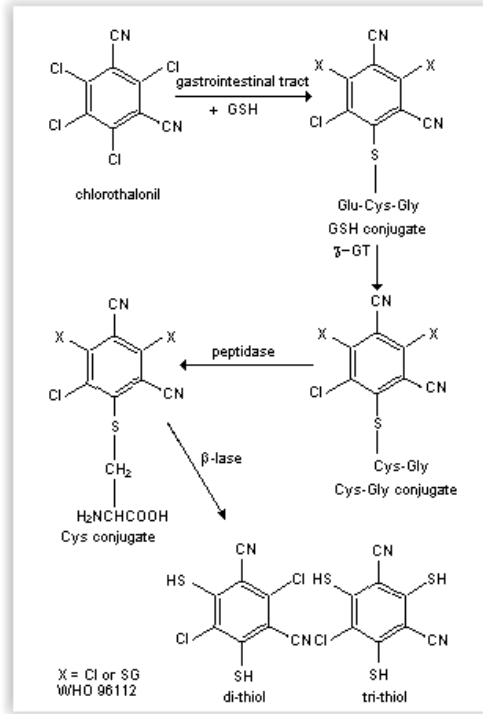
Renal cell regenerative proliferation

Is the weight of evidence sufficient to establish a mode of action (MOA)?

YES



Nephrotoxicity of Chlorothalonil in Rats



- 28-day study: Increased renal weight, LOAEL 80 mg/kg bw
- 90-day study: Increased BUN (NOAEL 40 mg/kg bw), increased renal weight (LOAEL 40 mg/kg bw), renal hyperplasia (LOAEL 40 mg/kg bw), karyomegaly in kidneys (LOAEL 40 mg/kg bw)
- 90-day study: increased renal weight (NOAEL **1.5 mg/kg bw**), hyperplasia of the epithelium of proximal convoluted tubules (NOAEL 10 mg/kg bw)
- Two-year study: Increased BUN and serum creatinine (NOAEL 3.8 mg/kg bw), increased renal weight (NOAEL 3.8 mg/kg bw), hyperplasia of the epithelium of proximal convoluted tubules (NOAEL **1.8 mg/kg bw**)

Carcinogenicity of Chlorothalonil in Rats

Renal adenomas + carcinomas

Dose (mg/kg bw per day)	Males	Females
116 week study		
0 (control)	0/60	0/60
40	7/60	3/60
80	7/60	6/60
175	19/60	23/60
2-year study		
0 (control)	1/55	0/55
1.8	1/54	0/54
3.8	1/54	0/55
15	4/54	0/53
175	23/55	32/55
Overall NOAEL: 3.8 mg/kg bw per day		

Hazard Characterisation of Chlorothalonil

- JMPR concluded that it is unlikely that chlorothalonil is genotoxic
- JMPR concluded that the formation of kidney tumours was the result of prolonged renal cytotoxicity and regenerative cell proliferation, and is consistent with a threshold phenomenon
- JMPR established an ADI for chlorothalonil of **0-0.02 mg/kg bw** based on a NOAEL of 1.8 mg/kg bw per day identified on the basis of kidney toxicity observed in long-term studies of toxicity in rats and using a safety factor of 100
- This ADI provides a **margin of 200** for the induction of renal tumours in rats (NOAEL 3.8 mg/kg bw per day)
- The Meeting concluded that, based on the MOA, while it is plausible that humans are less sensitive to the renal effects of chlorothalonil, it was not possible to dismiss relevance to humans on quantitative grounds, nor was it possible to quantify any difference in sensitivity
- However, given the species differences in the β -lyase bioactivation pathway, the ADI is likely to be conservative

Conclusions

- Cancer is often secondary to primary toxicity, i.e., through a non-genotoxic mode of action
- The key events are often reversible and will occur some time before the development of a carcinogenic response, usually at lower doses
- Hence a risk-based approach, based on overall weight-of-evidence, would be more than adequate to prevent cancer risk and would avoid unnecessary attrition and restrictions on otherwise useful substances



References

- Cohen et al (2019). Chemical carcinogenicity revisited 3: Risk assessment of carcinogenic potential based on the current state of knowledge of carcinogenesis in humans. *Regul Toxicol Pharmacol* 103:100-105
- EFSA CONTAM Panel (2011). Scientific Opinion on Tetrabromobisphenol A (TBBPA) and its derivatives in food. *EFSA Journal* 9:2477
- FAO/WHO (2009). JMPR: Pesticide residues in food – 2009, Part II- Toxicological. WHO, Geneva
- Meek et al (2015). New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. *J Appl Toxicol* 34:1-18.
- National Toxicology Program (2014). Tetrabromobisphenol A, NTP TR 587. NTP, Research Triangle Park, NC

