The Human Relevance Framework Applied to Dioxin's Mode of Action for Carcinogenicity

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Introduction

The Human Relevance Framework (HRF) provides a systematic examination of the postulated mode of action (MOA) for animal carcinogens and its relevance to humans[1,2]. The HRF-MOA evaluation also support the selection of the dose-response model applied in risk assessment. In the following abstract we briefly present a HRF-MOA case study for rodent tumors induced by aryl hydrocarbon (AHR) agonists, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin).

Methods and Materials

TCDD-induced liver tumors have been shown to be the most sensitive cancer outcome for dioxin cancer risk assessment[3,4,5]. Numerous cancer bioassays, initiation-promotion studies and mechanistic studies allow a robust examination of the MOA for dioxin-induced liver tumors. The HRF, specifically for dioxin-induced rodent liver cancer, is comprised of the following components.

1. *Postulated MOA: Key Events:* Measured biological events that underlie dioxin's rodent tumor MOA are identified
2. *Dose-response relationship:* Each key event's dose-response relationship with respect to other key events is described.
3. *Temporal associations:* The sequence of the key events leading to dioxin-induced rodent liver tumors is described.
4. *Strength, consistency and specificity of the association:* A complete review of all data especially with regards to their consistency across studies is examined.
5. *Biological plausibility and coherence:* The measured key events are examined against current models of liver cancer biology.
6. *Other MOAs:* Other MOAs, i.e., mutagenicity, are ruled-in or ruled-out.
7. *Conclusion:* The degree of confidence in the postulated MOA is provided.
8. *Uncertainties, inconsistencies, and data gap:* Deficiencies in the key events and postulated MOA are identified.

Results and Discussion

The postulated MOA for dioxin involves two MOA(s), depending on the dose administered (Figure 1). Starting early in the process, low dosages of TCDD induce a promotional stimulus within altered (spontaneously initiated) hepatic cells and foci. The key events underlying TCDD-induced tumor promotion involve activation of AHR pathways, inhibition of apoptosis and increased cell proliferation. At higher dosages and late time points (>6 months), toxic hepatopathy develops providing growth stimuli that drive mitosis thereby resulting in a regenerative repair MOA with increased cell division as a key event. A good example of TCDD-promotion of DEN-initiated altered hepatic foci, that demonstrates the dose-response aspects of this postulated MOA as well, was published by Pitot et al (1987) (Figure 2)[6]. Two examples of important key events,

inhibition of intrafocal apoptosis and increased cell proliferation (increase in foci volume) are shown in Figures 3 and 4[7,8]. While more data will be provided in the complete assessment of the HRF for dioxin-induced liver cancer, a number of concordance tables are shown below. The first concordance table demonstrates the relationship between dose-response and temporality for the key events, MOA and tumors. The second table provides a brief summary relating concordance evidence for the MOA and key event in laboratory animals and humans.

In summary, the use of the HRF framework is intended to objectively, completely and transparently present data that support the postulate MOA for dioxin-induced liver cancer. We conclude that the postulated MOA and key events, when examined with the HRF criteria, support the implementation of a threshold cancer model for dioxin risk assessment. Once established the MOA can be used to ask the question of relevance to humans and provide the basis for the dose-response model best supported for cancer risk assessment.

References

1. Meek, M.E., Bucher, J.R., Cohen, S.M., V. Dellarco, R.N. Hill, L.D. Lehman-McKeeman, D.G. Longfellow, T. Pastoor, J. Seed and D. E. Patton 2003 *Crit Rev Toxicol* 33: 591-653
2. Cohen, S.M., J. Klaunig, M.E. Meek, R.N. Hill, Pastoor, T., Lehman-McKeeman, L., Bucher, J., Longfellow, D.G., Seed, J., Dellarco,V., Fenner-Crisp, P. and Patton D. 2004 *Tox Sci* 78: 181-186
3. OEHHA DRAFT For Public Review Public Health Goal for TCDD In Drinking Water Prepared by Pesticide and Environmental Toxicology Branch Office of Environmental Health Hazard Assessment. California Environmental Protection Agency June 2007
4. U.S. EPA, 2003. U.S. Environmental Protection Agency, National Center for Environmental Assessment (NCEA), http://www.epa.gov/ncea/pdfs/dioxin/nas-review/.
5. Gray MN, Aylward LL, and Keenan RE. *J Toxicol Environ Health A*. 2006 69(10):907-17.
6. Pitot, H.C., Goldsworthy, T.L., Moran, S., Kennan, W., Glauert, H.P., Maronpot R.R. and Campbell, H.A. 1987 *Carcinogenesis* 8: 1491-1499
7. Stinchcombe, S., Buchmann, A., Bock, K.W., and Schwarz, M 1995 *Carcinogenesis* 16: 1271-1275
8. Teeguarden, J.G., Dragan, Y.P., Singh, J., Vaughan, J., Xu, Y.H. , Goldsworthy T. and. Pitot, H.C. 1999 *Tox Sci* 51: 211-223

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Figure 1: Postulated MOA and key events for both low and high dose response

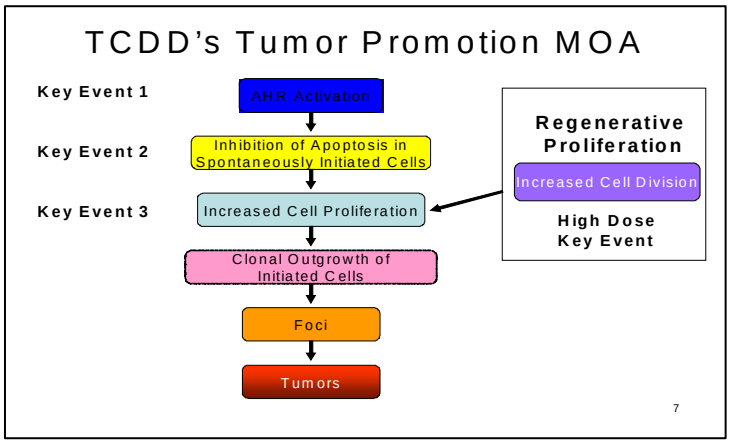


Figure 2: TCDD-inducedaltered hepatic foci following hepatectomy and initiation with diethylnitrosamine. Adapted from Pitot et al., 1987

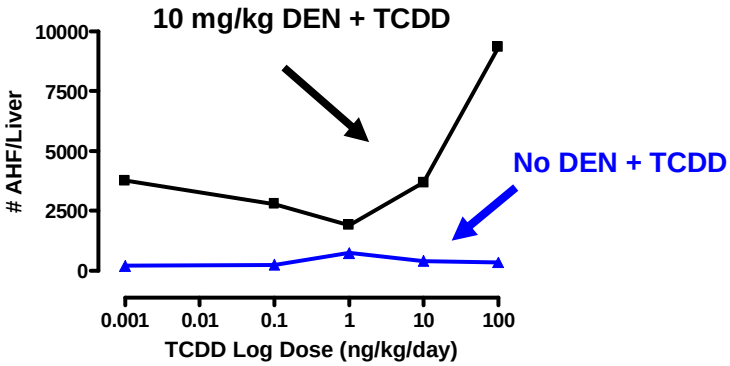
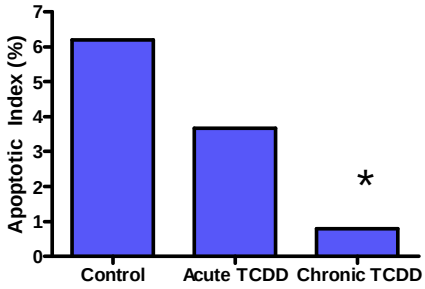


Figure 3: Intra-focal inhibition of apoptosis due to TCDD in DEN-initiated hepatic foci as adapted from Stinchcombe et al., 1995



Acute:
1.4 μg/kg x 1*
3 days prior to sacrifice

Chronic:
1.4 μg/kg biweekly x 9 wks

* $p < 0.05$

Figure 4: Evidence for TCDD-induced increased foci proliferation following DEN-initiation as adapted from Teeguarden et al., 1999.

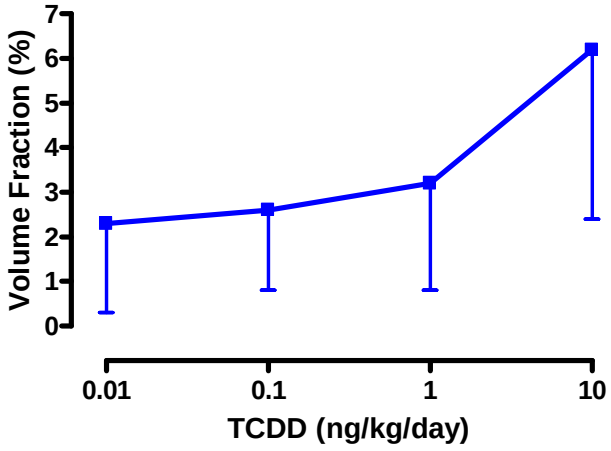


Table 1: Concordance between dose-response and temporality

Liver Conc. ng/kg	Temporality →					
	Promotion			Regenerative Proliferation		Liver Tumors
	AHR Activation	Apoptosis Inhibition	Cell Proliferation	Cell Division	Hepatopathy	
100-156	Yes	?	Yes	No	No	No
600		?		Yes	No	No
3000		Yes			No	No
3890					Yes	
5700						Yes

Table 2: Concordance between animals and humans

Key Event	Animal Evidence	Human Evidence	Qualitative Strength	Quantitative Strength
AHR Activation	Yes	Yes	Strong	Humans are less sensitive
Inhibition of Apoptosis	Yes	Plausible	Limited	Limited
Cell Proliferation	Yes	Plausible	Weak	None – No liver cancer in highly exposed workers
Cell Division	Yes	Plausible	Plausible	Transient elevation in liver enzymes