

DATE October 13, 1983

cc G.J. Levinskas - G2WF
G. Roush, M.D. - G2WG

SUBJECT Dioxin Seminar 10/11/83

REFERENCE

TO F.R. Johannsen - G2WD

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On Tuesday, October 11, Dr. Joyce Knutson of the McArdle Laboratory for Cancer Research, University of Wisconsin, presented a seminar describing in vivo and in vitro studies on the mechanism of 2,3,7,8-TCDD toxicity being conducted in her laboratory. Dr. Knutson works with Dr. A. Poland, a pioneer and expert in the area of dioxin's toxic mechanism(s). The following is a summary of Dr. Knutson's seminar.

The basic premise of her model, based on earlier work, was that the toxicity of TCDD and related compounds is mediated through binding to a receptor responsible for the induction of the cellular enzyme aryl hydrocarbon hydroxylase (AHH). For a tissue or cell type to develop a toxic response to chlorinated aromatic hydrocarbons the presence of the receptor is essential, but may not be sufficient since the ultimate biochemical lesion is still unknown. The model is supported by the results of two lines of experimental evidence: 1) Comparison of structure-activity relationships for potency to induce AHH activity and the toxic potency of these compounds, and 2) Comparison of the toxic susceptibility of inbred strains of mice which have either high-affinity or low-affinity receptors for AHH induction. Compounds with a high potency for AHH induction are more toxic, and mice with low-affinity receptors are less susceptible to the toxic action of these compounds.

Further work, however, revealed that differences in species sensitivity to AHH induction could not be accounted for by differences in either receptor affinity, receptor concentration, or pharmacokinetics (i.e., half-lives). They searched, therefore, for a toxic response which would correlate with AHH receptor affinity to investigate what other factors might be involved in the toxic action of TCDD and related compounds. The response identified was an epidermal hyperplasia and differentiation of the skin of genetically hairless ("nude") mice. The nude mice were sensitive to both AHH induction and the "hyperkeratosis" response when exposed to TCDD whereas their "haired" congenic counterparts, who were less sensitive to AHH induction, did not show the hyperkeratosis response. Using this in vivo system, they were able to demonstrate that TCDD is a tumor promoter but not an initiator.

From this work it was apparent that, as stated earlier, the presence of the TCDD/AHH receptor protein is essential, but not sufficient for eliciting a toxic response. To further investigate what might be occurring at the biochemical level, an in vitro cell

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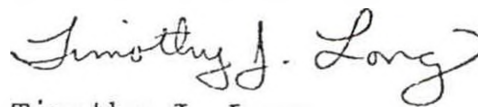
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culture system was derived that showed a keratinization response which correlated with receptor affinity as was seen in the in vivo situation. So far, using this system, they have found that the toxic response is not mediated via increases in cyclic nucleotides (i.e., cAMP) or changes in prostaglandin synthesis. Further work is now being conducted.

Thus, the current model of the mechanism of action of TCDD and related compounds, as I understand it, involves the binding of TCDD to a cytosolic receptor protein followed by translocation of this TCDD-receptor complex to the cell's nucleus. Here it interacts with the DNA, resulting in altered gene expression as evidenced by changes in enzyme activities and other responses such as altered cell division and differentiation. It is the latter effect which is felt to result in a toxic lesion.

Dr. Poland will be at Monsanto on Monday, October 17, to further discuss his work.


Timothy J. Long

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cc F.R. Johannsen

SUBJECT DIOXIN SEMINAR BY DR. JOYCE KNUTSON

REFERENCE

TO G. Roush
G.J. Levinskas

Fred Johannsen asked me to provide you with a summary of the referenced seminar which I attended on Oct. 11. Unfortunately, I did not take any notes, therefore my summary has the take home messages from this seminar rather than any specific data. The first part of a three part seminar consisted of a synopsis of the available toxicity data on 2,3,7,8-tetrachlorodibenzodioxin (hereafter referred to as TCDD). As has been pointed out numerous times before, Dr. Knutson stated that the guinea pig appeared to be most sensitive to the acute toxic effects of TCDD while the hamster was the least sensitive species. Another interesting observation was TCDD is a relatively late-acting toxin in that even at dosages 2-3 times the LD50, death generally occurs three to four days after compound administration.

The second part of the talk was discussion of the subcellular model for mechanism of dioxin toxicity. The investigators at the University of Wisconsin have postulated that TCDD and related halogenated aromatic hydrocarbons bind with a cytoplasmic receptor and this receptor ligand complex is translocated to the nucleus. Subsequent changes in the nucleus are responsible for both the induction of different cytochrome P-450 dependent enzymes and the toxic response. In support of this model, Dr. Knutson presented data which showed that the degree of binding of various chlorinated aromatic hydrocarbons to the cytoplasmic receptor correlated well with the degree of induction of liver enzymes as well as the acute toxic response in a given species. Using the hairless mouse as a model, Dr. Knutson has shown that skin keratinization can be produced with TCDD and other related compounds but not with other promoters like the phorbol esters. Liver enzyme induction also is observed in the hairless mouse and chlorinated compounds which lacked the ability to produce enzyme induction also did not induce skin keratinization.

The following two observations could not be explained totally on the receptor binding capabilities of these compounds.

- 1) The liver enzymes of a group of mice which differed from the hairless mice only at the genetic locus responsible for the hairless trait were inducible by TCDD but the skin of these animals did not show any keratinization when TCDD was applied to their shaved skin.

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- 2) The degree of binding with the cytoplasmic receptor is also unable to account for the vast differences in toxicity in various species. Thus, additional factors are needed to totally explain mechanism of toxicity of this compound.

The third part of the seminar provided information on use of a specific cell line (XB) for studying biochemical mechanisms of dioxin toxicity. XB cells normally plated at low density will form deep red/purple colonies but at a high density do not show this response. Dr. Knutson has shown that introduction of TCDD in XB cells plated at high density also develop these red/purple colonies. She defines this response in XB cells as keratinization and has shown that degree of keratinization following TCDD exposure is dose-dependent. Additional data presented showed that the keratinization is not mediated via cyclic AMP. Further studies to elucidate the mechanism of keratinization are in progress.

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