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Progress Report: Evaluation of In Vitro Assays for the Detection of "Dioxin-Like" Activity in the Binghamton State Office Building

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Progress Report: Evaluation of In Vitro Assays for the Detection of "Dioxin-Like" Activity in the Binghamton State Office Building. Gierthy, J.F. and Frenkel, G.D. (May, 1983) Previous reports have described the development and application of two in vitro systems for the detection of "dioxin-like" activity in extracts of soot contaminated with polychlorinated dibenzodioxins and dibenzofurans. The cell keratinization system, based on the induction of keratinization in epithelial cells by exposure to 2,3,7,8-TCDD, and the flat cell assay, based on a 2,3,7,8-TCDD induced change in cellular morphology have both been shown to be potentially useful in the detection of dioxin-like activity in extracts of soot from the Binghamton State Office Building (BSOB).

Further verification of the specificity of the flat cell induction to 2,3,7,8-TCDD exposure has been carried out. Various polychlorinated dioxins (PCDDs) and dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), polynuclear aromatic hydrocarbons (PAHs), and pesticides have been tested for their ability to induce the flat cell morphology. Results show that 2,3,7,8-TCDD is the most potent inducer of this morphological change, while the other PCDD and PCDFs have activity ranging from 10^{-1} to 10^{-3} of this isomer. The PCBs, PAHs and pesticides had lower activity ranging from 10^{-3} to less than 10^{-6} that of 2,3,7,8-TCDD. Where comparison was possible, a good correlation was seen between the published relative activity of compounds in the keratinization system and their activity in the flat cell assay. These results suggest that the sensitivity and specificity of the flat cell assay is similar to that of the keratinization system.

Previously, benzene extracts of cotton gauze swipes from the BSOB were found to contain a substance which precipitated during solvent exchange to DMSO and resulted in a loss of activity in the bioassays. This substance has now been found to be associated with the cotton gauze swipe pads. Results show that pre-extraction of the gauze pads with benzene allows full recovery of activity.

Hyperkeratinization is considered to be responsible for the occurrence of chloracne in humans exposed to polychlorinated dioxins and dibenzofurans (Poland et al., 1982). This effect is thought to be caused by an induced differentiation of the squamous epithelium. In this regard, 2,3,7,8-tetrachlorodibenzo(p)dioxin (2,3,7,8-TCDD) is considered to produce a sustained stimulation of a normal physiological response which leads to chloracne (Poland et al., 1982). Knutson and Poland have used the in vitro XB/3T3 cell keratinization system (Rheinwald et al., 1975) to study this effect of chlorinated dioxin isomers and congeners (Knutson et al., 1980). In previous reports (Gierthy et al., 1982a,b) we have demonstrated the use of this system as an assay for the detection of these compounds in Binghamton State Office Building (BSOB) soot extracts.

During these experiments, the appearance of an altered cell morphology was observed in those cells which had been exposed to 2,3,7,8-TCDD or the soot extracts (Gierthy et al., 1982a,b). This change was first seen after 7 days of exposure to the samples and was characterized by a flat cell morphology as compared to the more fusiform cells in the unexposed cultures or those exposed to less than 10^{-11} M 2,3,7,8-TCDD. An apparent cessation in cell growth was also seen in these cells, while the unexposed cells continued to proliferate, resulting in more dense cultures.

The minimal concentration of each soot sample extract capable of inducing the flat cell response was recorded as a measure of relative activity. The correlation between the concentration required for induction of the keratinization response and the flat cell morphology (Gierthy et al., 1982a) indicated that it may be possible to use the flat cell morphological change in these cells as an alternative and improved endpoint in the assay for dioxin-like activity.

The reproducibility of the assay as well as its ability to differentiate between various concentrations of 2,3,7,8-TCDD has been shown to be comparable to that of the cell keratinization system (Gierthy et al., 1982b). Other cell lines, including fibroblasts and transformed epithelial cells, were tested for their ability to respond to 2,3,7,8-TCDD with a change in morphology, but no flat cell effect was observed. Various inhibitors of macromolecular synthesis and mitosis were examined for their ability to induce flat cell morphological change. As in the keratinization system (Knutson et al., 1980), all were found to be inactive over a broad range of concentrations (Gierthy et al., 1982b), indicating that the effect is not associated with general toxicity but may be a specific effect, analogous to, and perhaps related to, keratinization. This possibility was supported by the finding that 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF) also induced the flat cell effect (Gierthy et al., 1982b), but had approximately one tenth the potency as 2,3,7,8-TCDD, which is the relative potency which has been reported for these two compounds in the induction of keratinization (Knutson et al., 1980).

This report describes further experiments which have been performed to establish the validity of the flat cell system as an assay for dioxin-like activity, as well as preliminary studies regarding the application of the in vitro assays to the detection of dioxin-like activity in surface swipe samples taken from the BSOB.

Results and Discussion

Further Validation of the Flat Cell Assay Studies on 24 chemicals, including polychlorinated dibenzo(p)dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons, and pesticides have indicated at least a 63 million

fold range in the potential of these compounds for inducing the flat cell effect (Table 1). These data show that the flat cell effect, like the keratinization reponse, is by far, most sensitive to the more toxic PCDDs and PCDFs with 2,3,7,8-TCDD being the most potent. Specifically, 1,2,4,7,8-penta-CDD, which lacks chlorination in one of the lateral positions of one of the benzene rings shows 100 fold less activity than 2,3,7,8-TCDD. 2,3,7,8-TCDF was shown to be the most potent PCDF tested and, as in the keratinization system (Knutson et al., 1980), had a flat cell inducing activity an order of magnitude lower than 2,3,7,8-TCDD. Other PCDFs tested gave a range of activity, with the hexa-CDF being more potent than the octa- or di-CDFs. The range of activity observed for the PCDDs and PCDFs tested, relative to 2,3,7,8-TCDD was about 1,000 fold. This is contrasted by the activity of the various PCBs tested. Here the flat cell inducing activity was 10^4-10^6 times less potent than 2,3,7,8-TCDD. Similar relative activity was reported in the keratinization system for another halogenated biphenyl, 2,3,4,2',3',4'-hexabromobiphenyl (Knutson et al., 1980). The polynuclear aromatic hydrocarbons varied in activity in relation to 2,3,7,8-TCDD. Dibenzo[a,h]anthracene and benz[a]anthracene were about 10^3 and 10^4 times less potent than 2,3,7,8-TCDD respectively. This correlates well with the reported activities of these compounds in the cell keratinization system (Knutson et al., 1980). 3-Methylcholanthrene and benzo(a)pyrene were both toxic at concentrations which were insufficient to induce a significant flat cell effect. These compounds were found to be inactive as inducers of keratinization in the XB/3T3 system (Knutson et al., 1980). The pesticides were all inactive in inducing the flat cell response at the concentrations tested with the exception of Mirex which had the highest

potency of this group (10^6 times less active than 2,3,7,8-TCDD). These data indicate that the specificity of the flat-cell assay appears similar to that of the cell keratinization system.

Application to BSOB Samples Versar New York Inc. (Springfield, Va.) has supplied twenty-nine benzene extracts of soot samples from the BSOB for preliminary testing of the bioassays. These included benzene extracts of cotton gauze swipes of BSOB surfaces, matrix blanks and reagent blanks. Upon solvent exchange into DMSO, a precipitate was formed in all of these samples except the reagent blank and therefore seemed to be associated with the gauze pad extracts. Furthermore when the matrix blanks and reagent blanks were spiked with 2,3,7,8-TCDD prior to solvent exchange, the activity of spiked matrix blank samples was significantly lower than that of spiked reagent blanks. The presence of a waxy precipitate was confirmed by similar solvent exchange performed by Versar on the cotton gauze swipes (Sonchik, 1982). Versar also noted that a PCB spike (Aroclor 1254, 1 µg/pad) partitioned into the precipitate, thus reducing the concentration found in the DMSO.

Versar subsequently supplied benzene extracts of gauze pads which had been pre-extracted with benzene. When these samples were solvent exchanged as before no precipitate was observed in three samples and only a very slight precipitate in one of the samples. Furthermore when these samples were spiked with 2,3,7,8-TCDD prior to solvent exchange, full bioassay activity was recovered. No bioassay activity was found when unspiked samples were tested.

From these results, it can be concluded that the precipitate which formed during solvent exchange of the benzene extract of the gauze swipes was a substance which had been extracted from the gauze. This precipitate

prevented full recovery of added 2,3,7,8-TCDD (as well as PCBs (Sonchik, 1982)). Pre-extraction of the cotton gauze pads with benzene effectively removes this substance. It appears that this procedure will allow further studies to be done concerning the feasibility of using the in vitro bioassays to detect dioxin-like activity in the surface swipe samples from the BSOB.

Table 1
Induction of the Flat Cell Effect by Various Chemicals

	Minimum Detectable Concentration (ppb)
2,3,7,8-Tetrachlorodibenzo(p)dioxin	0.0032
1,2,4,7,8-Pentachlorodibenzo(p)dioxin	0.359
2,3,7,8-Tetrachlorodibenzofuran	0.032
2,3,4,6,7,8-Hexachlorodibenzofuran	0.378
Octachlorodibenzofuran	4.48
2,6-Dichlorodibenzofuran	>2.38
3,4,3',4'-Tetrachlorobiphenyl	100
2,4,5,2',4',5'-Hexachlorobiphenyl	1,000
2,5,2',5'-Tetrachlorobiphenyl	>10,000
2,3,4,2',4',5'-Hexachlorobiphenyl	>10,000
2,3,4,2',3',4'-Hexachlorobiphenyl	>10,000
Aroclor 1254	10,000
Dibenzo[a,h]anthracene	10
Benz[a]anthracene	100
3-Methylcholanthrene	>100[a]
Benzo(a)pyrene	>100[a]
ß-Naphthoflavone	1,000
Pyrene	>10,000
Mirex	10,000
Dieldrin	>10,000
Aldrin	>10,000
o,p'DDT	>10,000
Lindane	>10,000
α-BHC	>200,000

a Toxic concentration was 1,000 ppb.

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