# *IN VITRO* CYTOTOXICITY OF HEAVY METALS, ACRYLAMIDE, AND ORGANOTIN SALTS TO NEURAL CELLS AND FIBROBLASTS

**ELLEN BORENFREUND AND HARVEY BABICH**

**Laboratory Animal Research Center**
**Rockefeller University**
**New York, New York**

*The cytotoxicity of neurotoxic agents was determined for a series of brain-derived cell types and compared with their toxic effects on BALB/c 3T3 fibroblasts, using the neutral red assay. Ranking of toxicants according to their potencies was the same for all cells tested and was in the order of methylmercury>cadmium> mercury> zinc>acrylamide. For a series of di- and triorganotins the ranking order was dibutyl▷diphenyl▷dibenzyl▷dipropyl▷ diethyl▷dimethyltin and triphenyl▷tribenzyl▷trimethyltin, respectively. The test was sensitive enough to detect structure activity relationships between the degree of toxicity and the hydrophobic characteristics of the agents tested.*

## INTRODUCTION

There has been a steady increase in the production of synthetic xenobiotics for industrial and commercial applications, such as diorganotins for use as antitoxidants in the stabilization of plastics and triorganotins for use as biocides. The eventual release and degradation of these substances into the environment has prompted concern about their harmful effects on human beings and the indigenous aquatic and terrestrial biota. The highly lipid soluble di- and triorganotins, for example, are potent neurotoxicants producing primarily central myelinopathy and neuronal changes, respectively (Watanabe, 1980; Chang et al., 1982; Mushak, 1982). Similarly, the widespread use of heavy metals has led to examination of their possible deleterious

1. Address correspondence to: Dr. Ellen Borenfreund, Laboratory Animal Research Center, Rockefeller University, 1230 York Avenue, New York, NY 10021.

2. Key words: fibroblasts, *in vitro*, neural cells, neurotoxic agents.

3. Abbreviations: $Be_2Sn$, dibenzyltin dichloride; $Be_3Sn$, tribenzyltin dichloride; $Bu_2Sn$, dibutyltin dichloride; $CdCl_2$, cadmium chloride; $CH_3HgCl$, methylmercuric chloride; DMEM, Dulbecco's Modified Eagle's Medium; $Et_2Sn$, diethyltin dichloride; FBS, fetal bovine serum; $HgCl_2$, mercuric chloride; $Me_2Sn$, dimethyltin dichloride; $Me_3Sn$, trimethyltin hydroxide; $Phe_2Sn$, diphenyltin dichloride; $Phe_3Sn$, triphenyltin hydroxide; $Pr_2Sn$, dipropyltin dichloride; $ZnCl_2$, zinc chloride.

effects on the central nervous system and their contribution to neurotoxic disease (Harada, 1978). Acrylamide, a well-known neurotoxic chemical, causes sensory and motor deficiencies in humans and animals by producing axonal degeneration (Spencer, 1979; Sickles, 1986). In general it appears that the availability of a sensitive *in vitro* assay for the detection of different toxic environmental agents and the suitability of such an assay for the determination of structure-activity relationships (SARs), and thus the possibility to predict biological effects within certain classes of compounds, would be most valuable.

We previously reported the development of a quantitative, spectrophotometric *in vitro* cytotoxicity assay based on the binding of neutral red, a supravital dye, to the lysosomes of viable cells which had previously been exposed to a broad spectrum of xenobiotics (Borenfreund and Puerner, 1984; 1985; 1986). Neutral red is a weakly cationic dye which enters the cell by nonionic diffusion where it binds by electrostatic bonds with anionic sites in the lysosomal matrix. Alterations of the cell surface or of lysosomal membranes brought about by toxic xenobiotics leads to lysosomal fragility and no uptake of the dye (Bitensky, 1963; Nemes et al., 1979). Using both cultured mammalian, as well as fish, cells as the targets (Babich et al., 1986; Babich and Borenfreund, 1987a), it was shown that this assay provided a sensitive procedure for the determination of cytotoxic effects for a model series of phenolics and toluenes which correlated with their log octanol/water partition coefficients (log P) and of divalent cationic metals which correlated with their chemical softness parameters (Babich and Borenfreund, 1987b). The present paper examined the application of this assay to the SARs of a series of di- and triorganotins and to the toxic effects of known neurotoxicants, such as acrylamide and the heavy metals, methylmercury, cadmium, and zinc. Both rat and human neural cell cultures and 3T3 fibroblasts served as target cells in order to compare their responses to known neurotoxic agents.

## METHODS

*Cells.* Brains from 3-day old neonatal rats were freed of meninges, minced into 2 mm fragments, washed with PBS and digested for 10 min in 0.5% trypsin-0.02% versene at 37° C with gentle agitation by a magnetic stirrer. The supernatant was decanted and the trypsin was inactivated by a 1:1 dilution with a mixture of 1 part F-12 and 1 part Dulbecco's minimum Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 units penicillin G, 100 $\mu$g streptomycin, and 1.25 $\mu$g fungizone per ml. Digestion of the remaining tissue fragments was repeated twice. Supernatants were combined, centrifuged 1,000 $\times$ g for 5 min and the pellet dispersed in F12-DMEM medium and seeded to 100 mm culture dishes. The remaining undissociated tissue was vigorously pipetted into the above medium and seeded to the culture dishes. After reaching confluence, the glial cells were trypsinized with a 0.125% trypsin-0.02% versene solution and passaged. Aliquots were frozen for storage and later use. Several subcultures were propagated in differentiation-inducing, defined serum-less medium

(DDM) to derive astroglial cell cultures as described (Bottenstein, 1985) with minor modifications. The BITSH medium consisted of biotin 10 $\mu$g/ml, insulin 10 $\mu$g/ml, transferrin 10 $\mu$g/ml, selenium 10 ng/ml, and hydrocortisone $1 \times 10^{-8}$ M in DMEM medium without serum. Propagation of a second set of subcultures was continued in F12-DMEM (1:1) with 10% FBS and subsequently referred to as glial cells. Glial cell cultures were used for experiments through the eighth passage, whereas DDM-induced differentiated astroglial cells were used only for 2-3 passages. Glial cells and astrocyte populations were identified morphologically. Cultures after growth in defined medium closely resembled those described by Bottenstein (1985) as typical glial cells or astrocytes. Other cell lines, such as the murine neuroblastoma cells ($N_2a$), human glioma cells (kindly provided by Dr. Joan Shapiro, Memorial-Sloan Kettering Cancer Center, New York, NY) and BALB/c 3T3 fibroblasts were propagated in DMEM containing 10% FBS, penicillin, streptomycin and fungizone, as described above.

*Test Agents.* Mercuric chloride ($HgCl_2$), methylmercuric chloride ($CH_3HgCl$), cadmium chloride ($CdCl_2$), zinc chloride ($ZnCl_2$), and acrylamide were obtained from Sigma Chemicals (St. Louis, MO).

The tin series which was examined for structure-activity relationships (SARs) included the diorganotins: diethyltin dichloride ($Et_2Sn$), dimethyltin dichloride ($Me_2Sn$), diphenyltin dichloride ($Phe_2Sn$), dibenzyltin dichloride ($Be_2Sn$), dipropyltin dichloride ($Pr_2Sn$) and dibutyltin dichloride ($Bu_2Sn$), and of the triorganotins: trimethyltin hydroxide ($Me_3Sn$), tribenzyltin chloride ($Be_3Sn$), and triphenyltin hydroxide ($Phe_3Sn$). The tin series was kindly provided by Dr. Frederick E. Brinckman, U.S. Department of Commerce, National Bureau of Standards, Gaithersburg, Maryland, and were of purity levels at least 97%. Stock solutions were prepared in aqueous or organic solvents. After appropriate dilutions the final concentration of solvent was kept at nontoxic concentrations and did not lead to precipitation of the test agents.

*Experimental.* BALB/c 3T3 fibroblasts ($9 \times 10^3$), neuroblastoma $N_2a$ ($1.5 \times 10^4$), human glioma ($3.5 \times 10^4$), rat glial ($1.7 \times 10^4$), and rat astroglial cells ($1.8 \times 10^4$) were seeded to 96-well tissue culture plates and allowed to form monolayers of about 60-70% confluency after 24 hours incubation. Test agents diluted into medium in a range of concentrations were added at 0.2 ml per well, with 4 or 8 replicates per concentration, and were incubated for 24 hours as described (Borenfreund and Puerner, 1984; 1985; 1986a). Briefly, medium was removed and 0.2 ml of fresh medium containing 50 $\mu$g/ml of the supravital dye, neutral red (NR), was added to each well with a multichannel pipette. Two wells at the top of the first row received medium without NR and served as blanks for subsequent spectrophotometric analysis. The dye-containing medium which had been prepared 24 hours earlier was centrifuged before use, to remove fine crystalline precipitates. After 3 hours of incubation, the NR medium was removed from the culture and the cells were rapidly washed with a mixture of 1% formalin-1% calcium chloride. The dye, bound to the

lysosomes of viable cells, was then extracted into the supernatant with 0.2 ml of a solution of 1% acetic acid-50% ethanol; the plate was placed for a few seconds on a plate shaker and 10 minutes later transferred to a microtiter plate reader where absorbance was measured at 540 nm and recorded. Data were expressed as percent of the relative, untreated control and presented either as 90% survival (NR-90), which was equivalent to the highest tolerated dose (HTD) (Borenfreund and Puerner, 1984; 1985), or as NR-50, the midpoint in the toxicity curve. For the tin series complete toxicity curves were presented and correlations established between NR-50 and the Hansch parameter values (Hansch and Leo, 1979) to demonstrate that toxicity of the series of organotins was related to their partitioning (hydrophobic) behavior and the chemical speciation products formed. Data represent 3-5 independent experiments and values were calculated as percent of relative control.

## RESULTS

As noted in Table 1 there were no striking differences between the various target cells with respect to their sensitivities to molar concentrations of mercury, methylmercury, cadmium, zinc, or acrylamide, although variation at the highest tolerated dose (HTD), which is equivalent to an NR-90, was somewhat greater than for the 50% lethality (NR-50) value.

On a molar basis methylmercury was the most toxic and acrylamide the least toxic agent to the various cell types examined. The general ranking in toxic potencies in

**TABLE 1**
**Toxicants Assayed *in Vitro***

| | NR-90 (in $\mu$M) | | | | |
|---|---|---|---|---|---|
| **Cell Type** | **Hg** | **$CH_3Hg$** | **Cd** | **Zn** | **Acrylamide** |
| Glial (rat) | 10 ± 1.2 | 1.5 ± 0.2 | 10 ± 1.0 | 120 ± 6.0 | 1500 ± 25.5 |
| Astroglial (rat) | 14 ± 1.8 | 0.6 ± 0.1 | 8 ± 0.5 | 140 ± 8.0 | NT |
| Neuroblastoma (murine) | 6 ± 0.4 | 0.4 ± 0.1 | 8 ± 0.8 | 100 ± 1.6 | 1260 ± 18.0 |
| Glioma (human) | 8 ± 0.8 | 1.0 ± 0.2 | 20 ± 1.5 | 120 ± 2.8 | 1260 ± 24.2 |
| Balb/c 3T3 fibroblast (murine) | 10 ± 0.8 | 2.0 ± 0.4 | 8 ± 0.4 | 100 ± 4.0 | 1400 ± 20.8 |
| | NR-50 (in $\mu$M) | | | | |
| Glial (rat) | 50 ± 3.0 | 5.0 ± 0.8 | 30 ± 2.2 | 180 ± 6.5 | 5600 ± 38.0 |
| Astroglial (rat) | 45 ± 2.5 | 4.5 ± 1.0 | 30 ± 2.0 | 200 ± 8.4 | NT |
| Neuroblastoma (murine) | 35 ± 0.8 | 3.0 ± 0.3 | 30 ± 0.4 | 160 ± 2.2 | 2800 ± 25.8 |
| Glioma (human) | 50 ± 2.0 | 5.0 ± 1.0 | 55 ± 3.5 | 180 ± 3.0 | 5600 ± 30.2 |
| Balb/c 3T3 fibroblast (murine) | 30 ± 2.0 | 6.0 ± 0.5 | 20 ± 1.8 | 150 ± 6.0 | 4220 ± 30.5 |

Cells were seeded to 96-well plates and 24 hr later incubated with a series of dilutions of toxicants for 24 hr as described in Methods. Complete toxicity curves were plotted. Data were based on 3-5 independent experiments and expressed as NR-90 (90% survival, highest tolerated dose) and NR-50 (midpoint toxicity); mean ± S.D.; neutral red assay.

order of the most to the least toxic, based on NR-50 values, was $CH_3Hg$>Cd>Hg >Zn>acrylamide. As can be seen from the ratio of NR-90 to NR-50 concentrations, the toxicity curve for zinc was considerably steeper than those of the other agents tested.

The *in vitro* cytotoxic concentrations for 50% lethality (NR-50) of the toxicants tested ranged for the different cell types from 3-6 $\mu$M for $CH_3Hg$ to 2.8-5.6 mM for acrylamide. In related studies an Aroclor-induced rat S-9 microsomal activating system was incorporated during the incubation, as described elsewhere with other toxicants (Borenfreund and Puerner, 1987; Babich and Borenfreund, 1987c). A significant decrease (about 30%) of cytotoxic potency of acrylamide was observed when such mixed-function oxidases were included in the incubation mixture in assays in which both 3T3 or $N_2a$ cells served as targets (data not shown) (Borenfreund and Puerner, 1987).

In a different series of experiments, the cytotoxic effects of a group of the structurally related neurotoxic organotins were examined. Comparing mouse fibroblasts (3T3) with mouse neuroblastoma cells ($N_2a$), it was noted that the diorganotins were, in absolute molar concentrations, considerably more toxic to neuroblastoma cells than to the mouse fibroblasts, whereas the sensitivities of the two cell types to the triorganotins were about the same (Table 2). However, the overall ranking in order of potencies of the various tin compounds was identical for both cell lines. Thus, ranking for diorganotins from most to least toxic was $Bu_2$>$Phe_2$>$Be_2$>$Pr_2$>$Et_2$>$Me_2$ (Fig. 1) and for the triorganotins it was $Ph_3$>$Be_3$>$Me_3$ (Fig. 2).

The cytotoxic concentration for the organotins examined *in vitro* with 3T3 cells ranged from 8 mM for dimethyltin to 0.08 mM for triphenyltin for the highest

**TABLE 2**
**Comparative Cytotoxicities of Organotins**[a]

| | NR-90 | | NR-50 | |
|---|---|---|---|---|
| **Organotins** | **3T3** | **$N_2A$** | **3T3** | **$N_2A$** |
| $Me_2Sn$ | 8.0 | 8.0 | 64 | 25.0 |
| $Et_2Sn$ | 6.0 | 2.0 | 18 | 6.0 |
| $Pr_2Sn$ | 3.0 | 0.2 | 8.0 | 1.8 |
| $Be_2Sn$ | 1.5 | 0.5 | 2.3 | 1.4 |
| $Phe_2Sn$ | 0.6 | 0.06 | 1.6 | 0.6 |
| $Bu_2Sn$ | 0.1 | 0.02 | 0.4 | 0.14 |
| $Me_3Sn$ | 0.8 | 0.8 | 5.0 | 6.0 |
| $Be_3Sn$ | 0.06 | 0.06 | 0.7 | 0.6 |
| $Phe_3Sn$ | 0.08 | 0.015 | 0.2 | 0.2 |

[a]In $\mu$M.

Balb/c 3T3 fibroblasts and $N_2A$ neuroblastoma cells were incubated with a series of dilutions of organotins as described and NR-90 and NR-50 cytotoxicity values determined in 3-5 independent experiments with the neutral red assay. Mean $\pm$ S.D. never exceeded 10%.

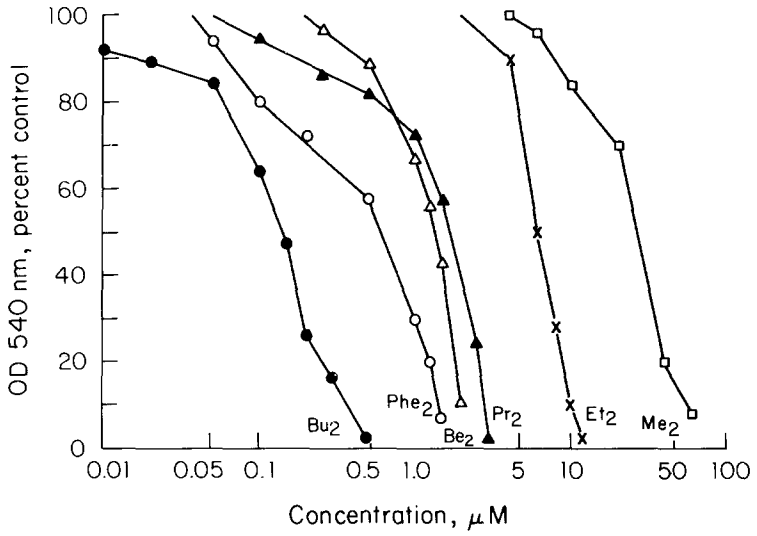


**FIGURE 1.** Cytotoxicity of diorganotin chlorides with $N_2a$ as target cells. $N_2a$ neuroblastoma cells were incubated for 24 hours with a series of dilutions (8 replicates per dilution) of diorganotins. Cytotoxicity was determined with the neutral red assay as described. Data were based on 3-5 independent experiments, mean ± S.D. agreed within 10%. $Bu_2$ = dibutyltin, $Phe_2$ = diphenyltin, $Be_2$ = dibenzyltin, $Pr_2$ = dipropyltin, $Et_2$ = diethyltin, $Me_2$ = dimethyltin.

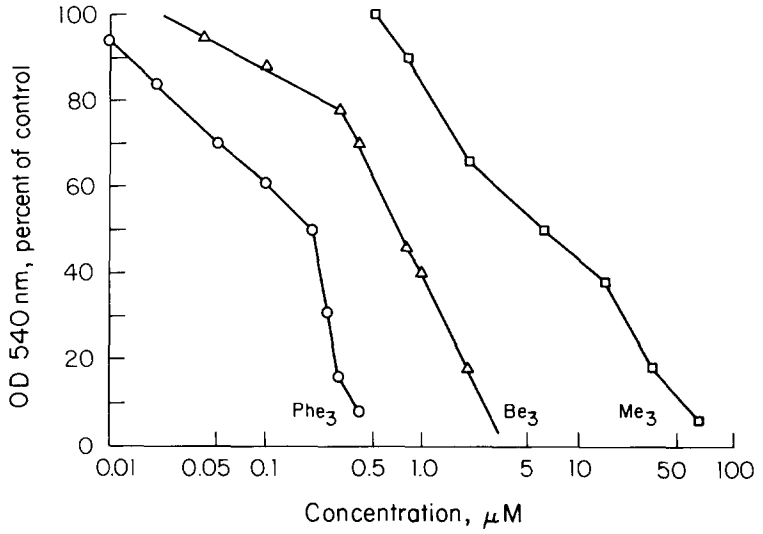


**FIGURE 2.** Cytotoxicity of triorganotin salts with $N_2a$ as target cells. $N_2a$ neuroblastoma cells were exposed to a series of dilutions (8 replicates per dilution) of triorganotins as described. Data were based on 3-5 independent experiments. Mean ± S.D. agreed within 10 percent. $Phe_3$ = triphenyltin, $Be_3$ = tribenzyltin, $Me_3$ = trimethyltin.

tolerated doses (NR-90) and from 64 $\mu$M for dimethyltin to 0.2 $\mu$M for triphenyltin for 50% lethality (NR-50) (Table 2). There was some overlap in the sensitivity range for trimethyltin with some of the diorganotins. The tribenzyl and triphenyltins were either more potent or as potent as the most toxic diorganotins (i.e., those with phenyl or butyl groups).

There was a direct correlation with the dialkyltins between the length of the carbon chain attached to the tin atom and the toxicity of the compound. Thus, the methyltin was less toxic than the ethyltin which in turn was less toxic than the propyl and the butyltins. This was in agreement with the *in vivo* observation that the hydrophobic character of a particular organotin could be correlated with its biological activity (Laughlin et al., 1985). Similarly the *in vitro* cytotoxicities of the diorganotins to both the 3T3 and $N_2a$ cell lines was correlated with their hydrophobicity as defined by their Hansch parameters (Fig. 3a,b). The number of triorganotins tested was too few to provide a meaningful plot of toxicity as a function of hydrophobicity.

## DISCUSSION

In the studies presented, a series of agents of known *in vivo* neurotoxic activity have been examined *in vitro* for their cytotoxic effects, using cultured cells of neural and fibroblastic origin. One aspect of the research presented herein was to demonstrate that when an *in vitro* cytotoxicity assay employs an endpoint of generalized cytotoxicity (such as membrane damage in the NR assay), the selection of a target cell is of secondary importance in the interpretation of the data. Thus, in these studies the ranking sequence of cytotoxicity was $CH_3Hg$> Cd>Hg>Zn>acrylamide, regardless of the indicator cell—whether it be a fibroblast or a neural cell. We have previously shown (Borenfreund and Puerner, 1986a) that with ten percent serum present in the medium, the test agents were less toxic than when one percent serum was used, which apparently reflected the adsorption of the xenobiotics to the serum proteins. However, the overall ranking of the toxic potencies of the test agents remained unchanged.

The much greater *in vitro* cytotoxicities of Cd and Hg than of Zn noted with the mammalian neural and fibroblastic cells (Table 1) has also been demonstrated with cultured bluegill sunfish BF-2 cells (Babich et al., 1986). Such differential cytotoxicities of Cd and Hg as compared with Zn have been correlated with the chemial softness parameters of the divalent cationic metals (Babich and Borenfreund, 1987b). The lipophilicity of $CH_3Hg^+$ towards the neural and fibroblastic cells probably accounted for its greater *in vitro* cytotoxicity than $Hg^{2+}$, as well as $Cd^{2+}$ and $Zn^{2+}$. Conversely, the low *in vitro* cytotoxicity of acrylamide may be related to its low lipophilicity (i.e., a log P value of $-1.55$, with diethyl ether as the solvent (Hansch and Leo, 1979)).

We were interested in examining the relationship between chemical structure and biological activity and the possible use of the neutral red assay for the detection of such relationships. Various attempts have been made to correlate structural characteristics of chemicals with biological events by using either hydrophobic characteristics (log P)

(Wang, 1982; Babich and Borenfreund, 1987a,b) or more recently, by applying a triparametric approach using the Hansch equation, which relates a biological response to lipophilic (log P), electronic (Pka), and steric ($^{1}X$) characteristics (Laughlin et al., 1985; Vighi and Calamari, 1985).

The data for a series of di- and triorganotins showed a relationship between toxicity and the length as well as the octanol/water partitioning characteristics of the organic ligand tested, as indicated by complete Hansch $\pi$ parameters (Fig. 3). Using thymidine incorporation, trypan blue exclusion, and chromium release as indicators of cytotoxicity in *in vitro* studies with rat thymocytes, Snoeij et al. (1986) also reported greater toxicity with the lipophilic tripropyltin and tributyltin chlorides than with the more water soluble trimethyltin and triethyltin chlorides.

The sequence of *in vitro* cytotoxicity for the di- and triorganotins that was noted with the BALB/c 3T3 (Table 2) and $N_2a$ (Fig. 1 and 2) cells was similar to that noted in *in vivo* acute toxicity studies with *Daphnia magna* (Vighi and Calamari, 1985), with zoeae of the mud crab, *Rhithropanopeus harrisii* (Laughlin et al., 1985), and with the alga, *Ankistrodesmus falcatus* (Wong et al., 1982) (Table 3). In the *in vitro* studies reported herein, as well as in the above-mentioned *in vivo* studies, the sequence of

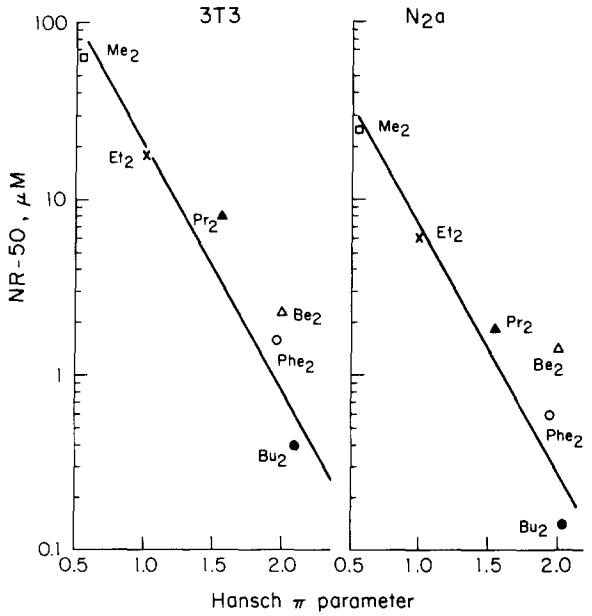


**FIGURE 3.** Cytotoxicity of diorganotin chlorides correlated with their lipophilicity (Hansch $\pi$ parameter). a) BALB/c 3T3 fibroblasts were incubated with diorganotins for 24 hours. Data were represented as NR-50 in $\mu$M and plotted against the hydrophobic characteristics of the toxicant as indicated by the Hansch $\pi$ parameter (Hansch and Leo, 1979). Abbreviations as in Fig. 1. b) $N_2a$ neuroblastoma cells were incubated and the data plotted as for 3T3 fibroblasts.

potency of the organotins was related to their lipophilicity, as correlated with their log P values or Hansch $\pi$ parameters. The lesser acute toxicity towards *R. harrisii* of $Be_2Sn$ than of $Et_2Sn$, $Pr_2Sn$, $Phe_2Sn$, and $Bu_2Sn$ (Table 3), was attributed to the instability of the parent compound during the 12-day exposure. Such a decomposition of $Be_2Sn$ into products of lesser toxicity was, apparently, not significant during the 1-day *in vitro* exposure of the 3T3 and $N_2a$ cell lines. Thus, in these *in vitro* cytotoxicity assays the potency of $Be_2Sn$ was more closely a function of its Hansch $\pi$ parameter.

The mechanism of $CH_3Hg$ toxicity *in vivo* is not yet understood. A high affinity for sulfhydryl groups, alterations of SH-containing macromolecules (Rothstein, 1973) and inhibition of brain protein synthesis (Amata, 1978; Syversen, 1982) which might account for the particularly high neurotoxicity to developing neural systems have been implicated. Acrylamide, which is believed to exert its neurotoxic effect either by inhibition of glycolytic enzymes (Spencer et al., 1979) or by inhibition of oxidative enzymes associated with energy transformation (Sickles and Goldstein, 1986) was found, on a molar basis, to be considerably less cytotoxic to all cell types than the other agents tested. We observed NR-50 toxic concentrations for $CH_3Hg$ to be 10 to 50 times greater than for Hg, Cd and Zn and 1000 × greater than for acrylamide. The toxic concentrations for acrylamide *in vitro* were similar to those reported by Ericsson and Walum (1986) in studies with rat glioma and mouse neuroblastoma cell lines.

The present series of studies, in which a number of neurotoxic agents have been examined under *in vitro* conditions, attest to the feasibility of testing toxicants under simple, controlled conditions and provide for an approach to investigate chemicals which might interfere with such toxicities. This assay furthermore allows for the prediction of cytotoxicities of agents heretofore untested within a series of structurally related chemicals.

**TABLE 3**
**Comparative Acute Toxicities of Diorganotins**

| | | *In Vitro* | | | *In Vivo* | |
|---|---|---|---|---|---|---|
| **Diorganotin** | **Hansch Parameter** | **3T3 $NR_{50}$, $\mu M$** | **$N_2a$ $NR_{50}$, $\mu M$** | **Mud Crab[1] $LC_{50}$, $\mu M$** | **Water Flea[2] $LC_{50}$, $\mu M$** | **Alga[3] $IC_{50}$, mg/L** |
| $Me_2Sn$ | 0.56 | 64.0 | 25.0 | 92.3 | 398.0 | 21.0 |
| $Et_2Sn$ | 1.02 | 18.0 | 6.0 | 14.6 | 15.8 | 16.0 |
| $Pr_2Sn$ | 1.55 | 8.0 | 1.8 | 14.0 | — | — |
| $Phe_2Sn$ | 1.96 | 2.3 | 1.4 | 2.6 | 1.9 | 8.0 |
| $Be_2Sn$ | 2.01 | 1.6 | 0.6 | 26.9 | — | — |
| $Bu_2Sn$ | 2.13 | 0.4 | 0.14 | 2.8 | 3.0 | 6.8 |

1. Laughlin et al. (1985); lethal concentration—50% values for a 12-day exposure of *Rhithropanopeus harrisii.*
2. Vighi and Calamari (1985); lethal concentration—50% values for a 1-day exposure of *Daphnia magna.*
3. Wong et al. (1982); primary productivity—50% values for a 1-day exposure of *Ankistrodesmus falcatus.*

## ACKNOWLEDGEMENT

This work was supported, in part, by Revlon, Inc.

## REFERENCES

BABICH, H., PUERNER, J.A. and BORENFREUND, E. (1986). *In vitro* cytotoxicity of metals to bluegill (BF-2) cells. Arch. Envrion. Contam. Toxicol. **14**:31–37.

BABICH, H. and BORENFREUND, E. (1987a). *In vitro* cytotoxicity of organic pollutants to bluegill sunfish (BF-2) cells. Environ. Res. **42**:229–237.

BABICH, H. and BORENFREUND, E. (1987b). Structure-activity relationship (SAR) models established *In vitro* with the neutral red cytotoxicity assay. Toxicol. *In vitro* **1**:3–9.

BABICH, H. and BORENFREUND, E. (1987c). Polycyclic aromatic hydrocarbon *in vitro* cytotoxicity to bluegill BF-2 cells; mediation by S-9 microsomal fraction and temperature. Toxicol. Lett., in press.

BITENSKY, L. (1963). The reversible activation of lysosomes in normal cells and the effect of pathological conditions. In: Ciba Foundation Symposium "Lysosomes" (A.V.S. de Reuck and M.P. Cameron, eds.), pp. 362–375, Little, Brown, and Co., Boston, MA.

BORENFREUND, E. and PUERNER, J.A. (1985). Toxicity determined *in vitro* by morphological alterations and neutral red absorption. Toxicol. Lett. **24**:119–124.

BORENFREUND, E. and PUERNER, J.A. (1987). Short-term quantitative *in vitro* cytotoxicity assay involving an S-9 activating system. Cancer Lett., in press.

BORENFREUND, E. and PUERNER, J.A. (1986b). Short-term quantitative *in vitro* cytotoxicity assay involving an S-9 activating system. Cancer Lett., in press.

BOTTENSTEIN, J.E. (1985). Growth and differentiation of neural cells in defined media. In: Cell Culture in the Neurosciences (J. E. Bottenstein and G. Sato, eds.), pp. 3–43. Plenum Publishing Corporation, New York, NY.

BOULDIN, T.W., GOINES, N.D., BAGNELL, C.R. and KINGMAN, M.R. (1981). Pathogenesis of trimethyltin neuronal activity, ultrastructural and cytochemical observations. Am. J. Path. **104**:237–248.

CHANG, L.W., TIEMEYER, I.M., WENGER, G.R., MCMILLAN, E.E. and REUHL, K.R. (1982). Neuropathology of trimethyltin interaction. Environ. Res. **29**:435–444.

CLARKSON, T.W. (1977). Mercury poisoning. In: Clinical Chemistry and Chemical Toxicology of Metals (S.S. Brown, ed.), pp. 189–200, Elsevier, North Holland, Amsterdam.

ERICSSON, A. and WALUM, E. (1984). Cytotoxicity of cyclophosphamide and acrylamide in glioma and neuroblastoma cell lines co-cultured with liver cells. Toxicol. Lett. **20**:251–256.

GATTI, G.L., MARCI, A. and SILANO, V. (1972). Methylmercury. In Trace Metals: Exposure and Health Effects. (Diferrante, E., ed.), pp. 73–98, Pergamon, Oxford.

HANSCH, C. and LEO, A.J. (1979). Substituent Constants for Correlation Analysis in Chemistry and Biology. John Wiley and Sons, New York, NY.

HARADA, M. (1978). Methylmercury poisoning due to environmental contamination. In: Toxicity of Heavy Metals in the Environment. (Oehme, F.D., ed.), pp. 261–302. Marcel Dekker, New York, NY.

LAUGHLIN, R.B. Jr., JOHANNESEN, R.B., FRENCH, W., GUARD, H. and BRINCKMAN, F.E. (1985). Structure-activity relationships for organotin compounds. Environ. Toxicol. Chem. **4**:343–351.

MUSHAK P., KRIGMAN, M.R., and MAILMAN, R.B. (1982). Comparative organotin toxicity in the developing rat. Somatic and morphological changes in relationship to accumulation of tin. Neurobehav. Toxicol. Teratol. **4**:209–215.

NEMES, Z., DIETZ, R., LUTH, J.B., GOMBA, S., HACKENTHAL, E., and GROSS, F. (1979). The pharmacological relevance of vital staining with neutral red. Experientia **35**:1475–1476.

OMATA, S., SAKIMURA, K., TSUBACK, H. and SUGANO, H. (1978). *In vivo* effect of methylmercury on protein synthesis in brain and liver of rats. Toxicol. Appl. Pharmacol. **44**:367–378.

ROTHSTEIN, A. (1973). Mercaptans, the biological targets for mercurials. In: Mercurials and Mercaptans (M.W. Miller and T.W. Clarkson, eds.), pp. 68–92. Thomas, Springfield, IL.

SICKLES, D.W. and GOLDSTEIN, B.D. (1986). Acrylamide produces a direct host-dependent and specific inhibition of oxidative metabolism in motor neurons. Neurotoxicology **7**:187–196.

SNOEIJ, N.J., VAN IERSEL, A.A.J., PENNINKS, A.H. and SEINEN, W. (1986). Triorganotin-induced cytotoxocity to rat thymus, bone marrow and red blood cells as determined by several *in vitro* assays. Toxicology **39**:71–83.

SPENCER, P.S., MOHAMMED, I., SCHAUMBERG, H.H. and MOORE, C.H. (1979). Does a defect of energy metabolism in the nerve fiber underlie axonal degeneration in polyneuropathies. Ann. Neurol. **5**:501–507.

SYVERSEN, T.L.M. (1982). Changes in protein and RNA synthesis in rat brain neurons after single doses of methylmercury. Toxicol. Lett. **10**:31–34.

VIGHI, M. and CALAMGRI, D. (1985). SARs for organotin compounds on *Daphnia magna*. Chemosphere **14**:1925–1932.

WATANABE, I. (1980). Organotins. In: Experimental and Clinical Neurotoxicology (P.S. Spencer and H.H. Schaumberg, eds.), pp. 545–557. Williams and Wilkins, Baltimore, MD.

WONG, P.T.S., CHAU, Y.K., KRAMAR, O. and BENGERT, G.A. (1985). Structure-toxicity relationship of tin compounds on algae. Can. J. Fish. Aquat. Sci. **39**:483–488.

Received December 15, 1986
Accepted February 25, 1987