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Transcriptional Profiles for Glutamate Transporters Reveal Differences Between Organophosphates but Similarities with Unrelated Neurotoxicants

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Transcriptional Profiles for Glutamate Transporters Reveal Differences between Organophosphates but Similarities with Unrelated Neurotoxicants

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Abstract: The developmental neurotoxicity of organophosphates involves mechanisms other than their shared property as cholinesterase inhibitors, among which are excitotoxicity and oxidative stress. We used PC12 cells as a neurodevelopmental model to compare the effects of chlorpyrifos and diazinon on the expression of genes encoding glutamate transporters. Chlorpyrifos had a greater effect in cells undergoing nerve growth factor-

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induced neurodifferentiation as compared to undifferentiated PC12 cells, with peak sensitivity at the initiation of differentiation, reflecting a global upregulation of all the glutamate transporter genes expressed in this cell line. In differentiating cells, chlorpyrifos had a significantly greater effect than did diazinon and concordance analysis indicated no resemblance in their expression patterns. At the same time, the smaller effects of diazinon were highly concordant with those of an organochlorine pesticide (dieldrin) and a metal (divalent nickel). We also performed similar evaluations for the cystine/glutamate exchanger, which provides protection against oxidative stress by moving cystine into the cell; again, chlorpyrifos had the greatest effect, in this case reducing expression in undifferentiated and differentiating cells. Our results point to excitotoxicity and oxidative stress as major contributors to the noncholinesterase mechanisms that distinguish the neurodevelopmental outcomes betweem different organophosphates while providing a means whereby apparently unrelated neurotoxicants may produce similar outcomes.

Keywords: Chlorpyrifos, Diazinon, Dieldrin, Gene transcription patterns, Glutamate transporters, Microarrays, Nickel, Organochlorine insecticides, Organophosphate insecticides, PC12 cells

Introduction

Organophosphates are the most widely-used insecticides but are undergoing increasing scrutiny because of their propensity to elicit developmental neurotoxicity [10,11,16,35,47]. As predicted by the results of laboratory studies over the past two decades, recent evaluations in human populations confirm a specific relationship between exposure to organophosphates and neurodevelopmental delays, depression and attention deficit hyperactivity disorder [6,7,24,42]. Originally, it was thought that these agents acted solely through inhibition of cholinesterase, so that exposure and toxicity could both be monitored readily by measuring the activity of this enzyme in plasma or red blood cells [36]. However, it is increasingly evident that organophosphates produce neurodevelopmental deficits below the threshold for signs of exposure or even without a detectable reduction in cholinesterase [11,47]. If organophosphates act through mechanisms other than their shared anticholinesterase properties, the various members of this class may differ in their ability to act as developmental neurotoxicants, and it then becomes important to identify the degree to which the different organophosphates target

those mechanisms. We recently showed that chlorpyrifos and diazinon, two of the most commonly-used organophosphates, differ substantially in their ability to elicit oxidative stress or excitotoxicity, with chlorpyrifos producing much larger changes in ionotropic glutamate receptor gene expression, whereas diazinon evoked greater effects on genes involved in apoptosis [50,54]. A subsequent paper pointed to further differences directed toward glutamate uptake and release and suggested the need to evaluate their comparative effects on glutamate transporters [44], the subject of the current study.

Our evaluations were conducted in PC12 cells, a widely-used in vitro model for neuronal development [64] that reproduces the mechanisms and outcomes of in vivo organophosphate exposures [3,4,13–15,18,25,26,33,37,39,40,49,50,59,60,62,66]. Nerve growth factor triggers differentiation of PC12 cells into neuronal phenotypes [20,62,64] and, like neurons, PC12 cells express the family of glutamate transporters that play an important role in the response to excitotoxic injury and oxidative stress, recapitulating the same functions as in the central nervous system [1,19,27,30,31,68]. Similarly, PC12 cells also possess the cystine/glutamate exchanger, which provides antioxidant protection by moving cystine into the cell in return for moving glutamate outwards [38].

For chlorpyrifos, we evaluated the effects in the undifferentiated state and during differentiation; we then compared the effects during differentiation with those of diazinon. Finally, we contrasted the effects of the organophosphates with those unrelated developmental neurotoxicants, the organochlorine insecticide, dieldrin, and a metal, divalent nickel. In our earlier work, we unexpectedly found similarities between their effects and those of organophosphates with regard to oxidative stress, excitotoxicity, cell signaling, neurotrophic responses and neurodifferentiation [2,50,53–57,60]. These additional test agents have intrinsic interest because of environmental concerns about human exposure and safety. Dieldrin is known to produce developmental neurotoxicity [8,28,29,34,50,67]; nickel shows fetal accumulation similar to that of lead [9,23] and shares neurotoxic actions with lead and cadmium [5]. For our evaluations, we used microarrays in a "planned comparisons" framework, where genes are selected based on a specific pathway and hypothesis prior to examining the microarray data, rather than the other way around; the

advantages and limitations of this approach as compared to searches of the entire genome have been presented previously [52,56,58–60]. Here, we restricted our examination to the glutamate transporter family. In addition to examining up- and downregulation of the glutamate transporter genes, we also assessed concordance between pairs of agents to determine overall similarities of the transcriptional responses [2,54,60].

Methods

Cell cultures

Because of the clonal instability of the PC12 cell line [20], the experiments were performed on cells that had undergone fewer than five passages. As described previously [41,62], PC12 cells (American Type Culture Collection, CRL-1721, obtained from the Duke Comprehensive Cancer Center, Durham, NC) were seeded onto poly-D-lysine-coated plates in RPMI-1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% horse serum (Sigma Chemical Co., St. Louis, MO), 5% fetal bovine serum (Sigma), and 50 μg/ml penicillin streptomycin (Invitrogen). Incubations were carried out with 7.5% CO_2 at 37°C, standard conditions for PC12 cells. To initiate neurodifferentiation [25,50,64] twenty-four hours after seeding, the medium was changed to include 50 ng/ml of 2.5 S murine nerve growth factor (Invitrogen). Along with the nerve growth factor, we added 30 μM of each of the test agents: chlorpyrifos (Chem Service, West Chester, PA), diazinon (Chem Service), dieldrin (Chem Service) or $NiCl_2$ (Sigma). The concentration was chosen from earlier studies that demonstrated adverse effects on differentiation of PC12 cells without outright cytotoxicity [26,39,50,59]. Because of the limited water solubility of the three insecticides, these agents were dissolved in dimethylsulfoxide (final concentration 0.1%), which was also added to the control cultures and to cultures containing $NiCl_2$; this concentration of dimethylsulfoxide has no effect on PC12 cell growth or differentiation [39,41,62]. Cultures were examined 24 and 72 hr after commencing exposure, with 5–8 independent cultures evaluated for each treatment at each time point. Each culture was run on a separate array. We used two time points so as to be able to evaluate changes in gene expression regardless of whether the mRNA for a given gene has

a rapid turnover (and hence can rise rapidly) or a slower turnover that would require a longer period to show corresponding increases or decreases. For chlorpyrifos, we evaluated the effects both on undifferentiated cells (without addition of nerve growth factor) and during differentiation, whereas for the other agents, we studied the effects only during differentiation.

Microarray determinations

Our earlier studies detailed the techniques for mRNA isolation, preparation of cDNA, conversion to cRNA incorporating cyanine-3 (reference RNA) or cyanine-5 (sample RNA), verification of RNA purity and quality, hybridization to the microarrays, washing and scanning [52,58,59]. These all involve commercial kits and standardized procedures, and since the current studies were done identically, the techniques will not be described here. The mRNA used for the reference standard was created by pooling aliquots from each of the samples in the study so as to ensure measurable levels of all genes expressed over the background. Array normalizations and error detection were also carried out by standard procedures described previously [52,58,59]. We used Agilent Whole Rat Genome Arrays (Agilent Technologies, Palo Alto, CA), type G4131A for the studies of chlorpyrifos in undifferentiated and differentiating cells, whereas type G4131F was used for the studies of diazinon, dieldrin and Ni^{2+} in differentiating cells. The two chips contain exactly the same gene sequences but the latter has a lower detection threshold; however, all the genes reported here passed the quality control filters with both arrays.

For several of the genes, the arrays contain multiple probes and/or replicates of the same probe in different locations on the chip, and these were used to verify the reliability of values and the validity of the measures on the chip. In these cases, to avoid artificially inflating the number of genes utilized for analysis, we limited each gene to a single set of values, selecting those obtained for the probe showing the smallest intragroup variance. The other values for that gene were used only to corroborate direction and magnitude of change. We also validated the readings on the arrays through the use

of duplicate arrays for one sample selected from each treatment group [52,58].

Statistical procedures

Because of the requirement to normalize the data across arrays, the absolute values for a given gene are meaningless, so only the relative differences between treatments can be compared. Our design involved planned comparisons of four agents at two time points, as well as the effects of one agent (chlorpyrifos) in undifferentiated vs. differentiating states. It was therefore important to protect against the increased probability of type 1 errors engendered by repeated testing of the same data base. Accordingly, before looking at effects on individual genes, we performed a global ANOVA incorporating all the variables in a single comparison: treatment, time, and all genes. Lower-order ANOVAs on subdivisions of the data set were then carried out as permitted by the interactions of treatment with the other variables. Differences for individual treatments for a specified gene at a single time point were evaluated with Fisher's Protected Least Significant Difference. However, for a given gene where there was no interaction of treatment with other variables (time, differentiation state), only the main treatment effect was reported without subtesting of effects at a single time point. Treatment effects were considered significant at $p < 0.05$ (two-tailed, since we were interested in both increases and decreases in gene expression).

To compare overall patterns of effects on gene expression, as distinguished from just identifying individual genes targeted by the treatments, we evaluated concordance between treatments by plotting the percentage change from control and calculating the linear correlation coefficient between pairs of agents [2,54,60].

Results

The microarrays revealed significant expression of eight glutamate transporters that passed the quality control procedures (Table 1), including those related to neurons (*slc1a1*), synaptic vesicles (*vglut3*) and glia (*slc1a2*, *slc1a3*), as well as those transporting glutamate into multiple cell types (*slc1a7*), glutamate as

a neutral amino acid (*slc1a4, slc1a5*) or aspartate/glutamate (*slc1a6*). In addition, we were able to measure expression of the cystine/glutamate exchanger (*slc7a11*), which we considered separately from the glutamate transporter family. Because only one agent (chlorpyrifos) was tested in both undifferentiated and differentiating cells, we conducted two sets of global statistical tests. For chlorpyrifos, the ANOVA factors were treatment, differentiation state, time and gene, and we found a main treatment effect ($p < 0.007$) as well as interactions of treatment × differentiation state × time ($p < 0.03$), treatment × time ($p < 0.04$) and treatment × time × gene ($p < 0.02$). Accordingly, we subdivided the results for presentation according to differentiation state and performed lower-order tests to identify main effects and interactions with the remaining variables (time, gene). Diazinon, dieldrin and Ni^{2+} were studied only in differentiating cells, so the ANOVA factors for these agents were treatment, gene and time. The global test identified significant interactions of treatment × time ($p < 0.02$) and treatment × gene × time ($p < 0.009$), so we subdivided the data into the individual treatments for presentation and lower-order tests.

Table 1
Genes encoding proteins involved in glutamate transport.

Gene	Genbank accession number	Description
slc1a1	NM_013032	Solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1
slc1a2	AY069978	Solute carrier family 1 (glial high affinity glutamate transporter), member 2
slc1a3	NM_019225	Solute carrier family 1 (glial high affinity glutamate transporter), member 3
slc1a4	NM_198761	Solute carrier family 1 (glutamate/neutral amino acid transporter), member 4
slc1a5	NM_175758	Sodium-dependent neutral amino acid transporter ASCT2
slc1a6	NM_032065	Solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6
slc1a7	XM_345559	Solute carrier family 1, member 7
vglut3	NM_153735	Vesicular glutamate transporter 3
slc7a11	XM_227120	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 11

In undifferentiated cells, chlorpyrifos elicited selective changes in the expression of glutamate transporter genes (Figure 1). There was a robust but transient increase in *slc1a3* and smaller, but more sustained increases for *slc1a4* and *slc1a5,* whereas *vglut3* was decreased. The pattern was quite different for cells undergoing differentiation (Figure 2A). Here, chlorpyrifos evoked a significant, global upregulation (main treatment effect) that was significantly greater at 24h of exposure than at 72h (treatment × time interaction) but that was nevertheless statistically significant at either of the time points. Diazinon had a much smaller effect on glutamate transporter gene expression than did chlorpyrifos (Figure 2B). There was no global, main treatment effect but rather, there were smaller, gene- and time-specific changes confined to *slc1a4* (transient increase),

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slc1a5 (small but consistent increase) and *vglut3* (decrease at 72h). Likewise, dieldrin (Figure 2C) had only small effects limited to a single significant change (*slc1a5*), as did Ni^{2+} (Figure 2D), which affected the same gene.

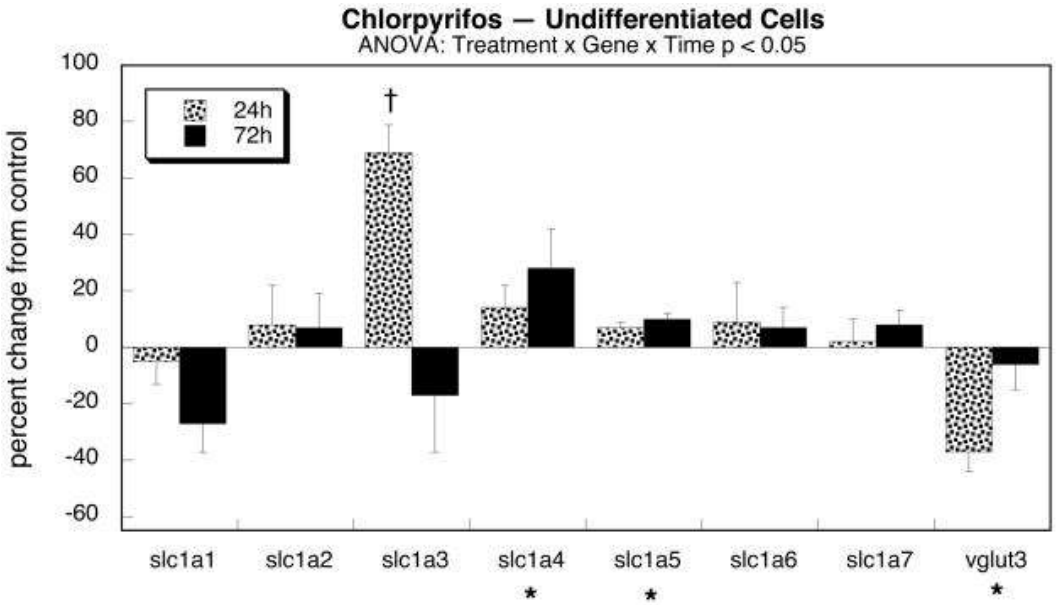

Figure 1 Effects of chlorpyrifos exposure on expression of genes for glutamate transporters in undifferentiated PC12 cells. Multivariate ANOVA appears at the top of the panel and asterisks shown below each gene denote a significant main treatment effect; daggers denote genes for which a treatment × time interaction was detected and show the individual times for which treatment effects were present. Expression ratios in the control group were: *slc1a1,* 1.12 ± 0.05 at 24h, 1.25 ± 0.06 at 72h; *slc1a2*, 1.04 ± 0.08 at 24h, 0.99 ± 0.12 at 72h; *slc1a3*, 0.72 ± 0.06 at 24h, 1.28 ± 0.09 at 72h; *slc1a4*, 0.80 ± 0.07 at 24h, 0.96 ± 0.08 at 72h; *slc1a5*, 0.84 ± 0.01 at 24h, 0.90 ± 0.01 at 72h; *slc1a6*, 0.86 ± 0.07 at 24h, 1.03 ± 0.06 at 72h; *slc1a7*, 0.99 ± 0.06 at 24h, 1.04 ± 0.06 at 72h; *vglut3*, 1.18 ± 0.09 at 24h, 1.05 ± 0.10 at 72h.

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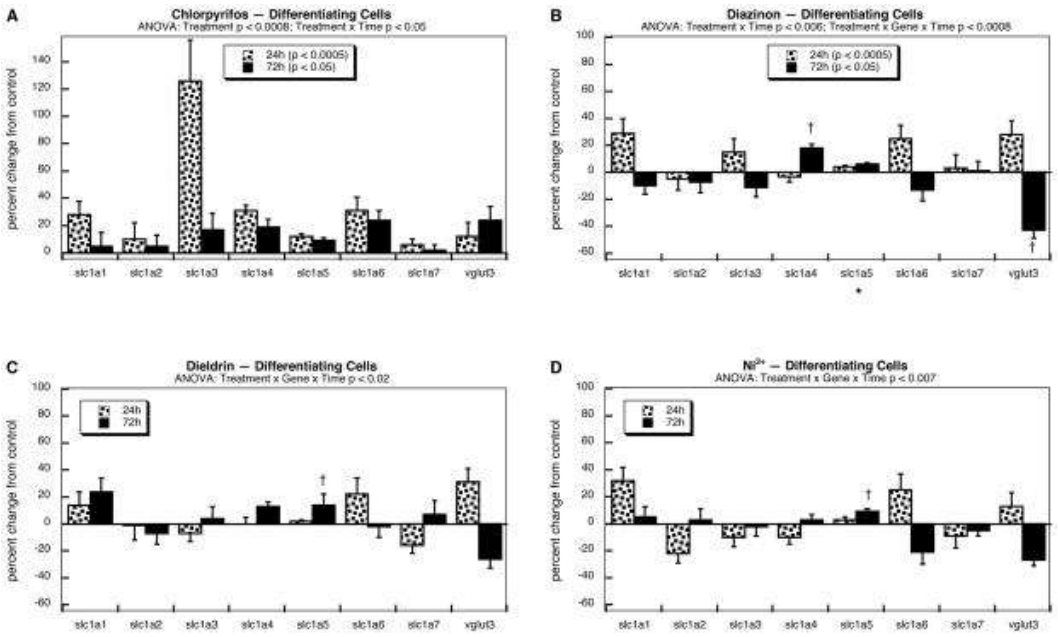

Figure 2 Effects of different neurotoxicants on genes for glutamate transporters in differentiating PC12 cells: (A) chlorpyrifos, (B) diazinon, (C) dieldrin, (D) Ni^{2+}. Multivariate ANOVA appears at the top of each panel. Where there was a significant difference in the treatment effects on the various genes (B,C,D), asterisks shown below each gene denote a significant main treatment effect; daggers denote genes for which a treatment × time interaction was detected and show the individual times for which treatment effects were present. Where there was only a treatment effect and interaction of treatment × time (A), the treatment effects for each time are shown within the legend box. Expression ratios in the control group were: *slc1a1,* 0.79 ± 0.08 at 24h, 0.94 ± 0.10 at 72h; *slc1a2*, 1.16 ± 0.09 at 24h, 1.36 ± 0.10 at 72h; *slc1a3*, 0.57 ± 0.06 at 24h, 0.95 ± 0.08 at 72h; *slc1a4*, 0.83 ± 0.09 at 24h, 1.09 ± 0.08 at 72h; *slc1a5*, 1.13 ± 0.04 at 24h, 0.99 ± 0.03 at 72h; *slc1a6*, 0.87 ± 0.09 at 24h, 0.84 ± 0.02 at 72h; *slc1a7*, 0.98 ± 0.03 at 24h, 0.96 ± 0.04 at 72h; *vglut3*, 1.12 ± 0.06 at 24h, 0.93 ± 0.08 at 72h.

To verify that the greater effects of chlorpyrifos in the differentiating cells were not solely dependent on the one determination showing the largest increase (*slc1a3* at 24h), we performed paired comparisons using the Wilcoxon signed-rank test so as to give equal weight to the differences between treatments for each gene regardless of the absolute magnitude of the individual effect. Chlorpyrifos exposure in differentiating cells had a greater net effect than in undifferentiated cells ($p < 0.008$). Similarly, chlorpyrifos had a greater effect than diazinon ($p < 0.005$), dieldrin ($p < 0.03$) or Ni^{2+} ($p < 0.003$), whereas comparisons among the other three agents showed no significant distinction from each other. We further examined

similarities and differences among the treatments by evaluating the pairwise concordance across all the gene and time measurements; this procedure assesses patterns of effects incorporating all genes and time points, regardless of whether the effects were individually significant. Although there was a significant correlation between effects of chlorpyrifos in undifferentiated vs. differentiating cells, the entire relationship depended on a single measurement out of the 16 values, making it unlikely that the responses were biologically related (Figure 3A). Similarly, there was no detectable relationship between the pattern of effects for chlorpyrifos in differentiating cells vs. either diazinon (Figure 3B), dieldrin (Figure 3C) or Ni^{2+} (Figure 3D). On the other hand, there was highly-significant concordance between the effects of diazinon and dieldrin (Figure 4A), diazinon and Ni^{2+} (Figure 4B) and dieldrin and Ni^{2+} (Figure 4C).

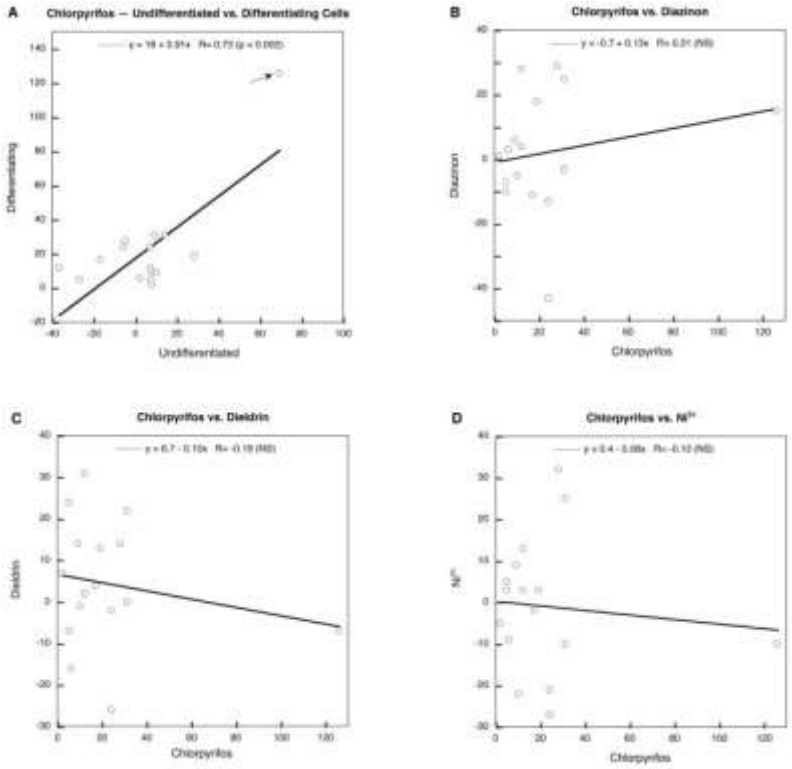

Figure 3 Pairwise correlations of the effects of chlorpyrifos in undifferentiated vs. differentiating cells (A), and in differentiating cells for chlorpyrifos vs. diazinon (B), dieldrin (C) or Ni^{2+} (D). In (A), a single point (*slc1a3* at 1h, arrow) is responsible for the significant correlation; without that point, R = 0.20, not significant (NS).

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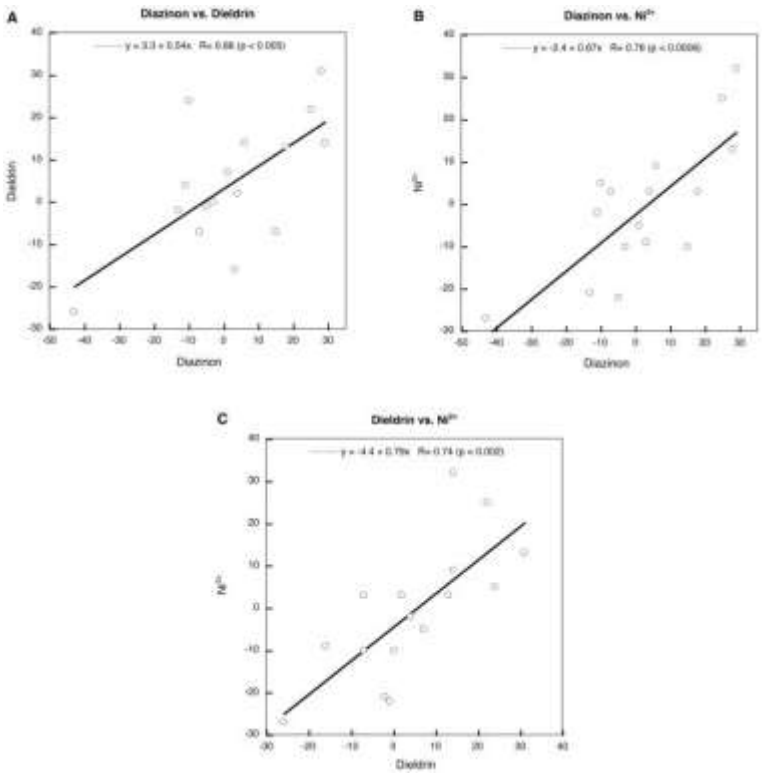

Figure 4 Pairwise correlations of the effects of diazinon vs. dieldrin (A), diazinon vs. Ni^{2+} (B) and dieldrin vs. Ni^{2+} (C) in differentiating cells. Linear correlation coefficients are shown at the top of each panel along with the least-squares fit.

Chlorpyrifos also had greater effects than the other agents toward expression of *slc7a11*, the cystine/glutamate exchanger (Figure 5). For this gene, undifferentiated cells showed strong and persistent suppression by chlorpyrifos whereas the differentiating cells were affected to a smaller, but still significant degree. None of the other agents suppressed *slc7a11* expression and indeed, dieldrin evoked a significant increase at 72h.

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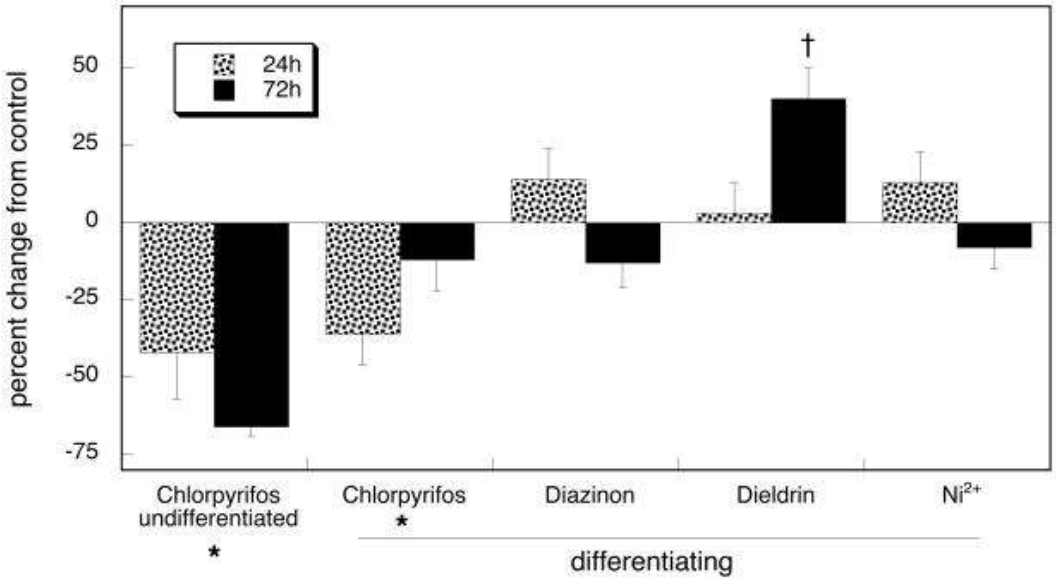

Figure 5 Effects of neurotoxicants on expression of the cystine/glutamate exchanger, *slc7a11*. Multivariate ANOVA indicates a significant main treatment effect ($p < 0.0007$) and a treatment × time interaction ($p < 0.02$). Asterisks denote treatments showing a main effect compared to control; the dagger denotes a significant treatment × time interaction and shows the individual time for which the treatment effect was present. Expression ratios in the control group were: undifferentiated cells, 2.09 ± 0.10 at 24h, 1.43 ± 0.15 at 72h; differentiating cells, 1.47 ± 0.12 at 24h, 0.92 ± 0.09 at 72h.

Discussion

Results obtained in this study reinforce the idea that, despite their shared property as cholinesterase inhibitors, organophosphates can differ substantially in their ability to evoke developmental neurotoxicity through noncholinesterase mechanisms [10,12,22,47]. Here, we identified effects involving the glutamate transporters found in the synaptic cell membrane (the seven *slc1a* genes) and that are thus responsible for removing extracellular glutamate, as well as one of the vesicular transporters (*vglut3*) that moves glutamate from the cytoplasm into synaptic vesicles; these transporters are among the most important factors that limit excitotoxicity resulting from glutamate release. We found a much greater effect of chlorpyrifos than diazinon, a result is in keeping with distinctions between the two organophosphates in their impact on ionotropic glutamate receptors [54] and in the response to NMDA receptor antagonists, which protect cells from chlorpyrifos but not diazinon [44]. It is particularly notable

that the effects of chlorpyrifos on glutamate transporters was greatest during the early stages of neurodifferentiation; in our previous work with ionotropic glutamate receptors, we found greater sensitivity for undifferentiated PC12 cells. This dichotomy implies that chlorpyrifos-induced excitotoxicity is likely to involve an extended range of developmental stages but with different underlying mechanisms for each stage. Indeed, the existence of such multiple mechanisms is one of the reasons that the developing brain is vulnerable to disruption by chlorpyrifos virtually throughout development, from the neural tube stage through late synaptic modeling and gliogenesis [46,47].

Although chlorpyrifos caused global upregulation of glutamate transporter gene expression, it downregulated the gene encoding the cystine/glutamate exchanger (*slc7a11*) in both undifferentiated and differentiating PC12 cells, but with greater effects in the former. Clearly, then, this involves a distinctly different mechanism from that mediating the effect on the transporters and more closely resembles the prior results for ionotropic glutamate receptors [54]. The cystine/glutamate exchanger moves cystine into the cell and glutamate out, with the subsequent intracellular formation of glutathione providing an important component of antioxidant defense. Organophosphates elicit oxidative stress as one of their noncholinesterase mechanisms of neurotoxicity [21,22,50,54,57] and hence, suppression of the exchanger could easily impair the ability of neuronal cells to withstand exposure. We are currently examining the function of the exchanger in primary mouse cortical cultures and, as predicted from the transcriptional effects seen here, our preliminary data indicate a clear-cut suppression of activity in response to an otherwise nontoxic, 24h chlorpyrifos exposure, (17 ± 4 percent reduction in [^{14}C]cystine uptake, $p < 0.006$, n=20). Impaired cystine/glutamate exchange is particularly important in the developing brain because it has lower reserves of antioxidants [22], and is deficient in glia, which ordinarily protect neurons from oxidative molecules [63], while facing the higher metabolic demand associated with growth. Further, the fetal environment is hypoxic relative to that of the neonate or adult [17,32], thus fostering the conditions for oxidative stress. Again, chlorpyrifos was far more active than diazinon in downregulating the cystine/glutamate exchanger, potentially exacerbating the consequences of its effects on oxidative stress, as well as on glutamate release and transport.

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Although it might be expected that chlorpyrifos and diazinon would be the most similar of the four toxicants evaluated here, we actually found that the effects of diazinon more closely resembled those of dieldrin and Ni^{2+}. For the glutamate transporters, there was highly-significant concordance among diazinon, dieldrin and Ni^{2+} but not for any of these agents with chlorpyrifos. This continues a pattern that we identified in earlier studies comparing organophosphates to other toxicants with regard to oxidative stress, cell signaling cascades, neurotrophins and their receptors and neurodifferentiation endpoints [2,50,53–57,60]. The conclusion is inescapable: apparently unrelated developmental neurotoxicants can nevertheless converge on quite similar downstream mechanisms of neurotoxicity, despite any underlying differences in their chemical composition or initial mechanism. In turn, this implies that any single specific mechanism of action may be less important for neurotoxic outcomes than examination of downstream events known to participate in neurotoxicity. At the same time, it should then be possible to design countermeasures that can ameliorate or prevent neurotoxicity from otherwise disparate neurotoxicants by focusing on the downstream targets.

In this study, we used planned comparisons of specific pathways targeted by the neurotoxicants and analyzed the data through the determination of shared properties (i.e. a standard "principal components" approach); the rationale for this has appeared previously [52,58,59] but is worth repeating here. Planned comparisons and pathway analysis are distinct from the use of microarrays to find a handful of genes that are affected the most, within the global examination of the tens of thousands of genes present on the microarrays. Planned comparisons are based instead on testing a specific hypothesis that centers around a defined set of genes, and rests on known, validating outcomes from prior work, in this case for the organophosphates. With examination of the entire genome, verification via RT-PCR and other techniques is required because the enormous number of comparisons generates many false positive findings (e.g. the >2000 genes that would be false positives if we had considered all 42,000 probes on the array). For our study, we compared only a few genes that would generate less than a single false positive, and for interpretation, we relied on concordance patterns to evaluate the overall spectrum of multiple gene changes for

each agent, rather than changes in any one gene; further, we made sure that effects were repeated across different treatments and/or different times; even for individual genes, there were multiple probes and multiple spots on a given array (see Methods), so the changes cannot be "chance." Indeed, one of the key points of this study is to demonstrate that a planned comparisons approach may provide a superior strategy for the use of microarray data, provided that the relevant target pathways can be selected in advance, based on specific hypothesis and prior data.

Our findings strengthen the view that, despite their common characteristic as cholinesterase inhibitors, organophosphates differ in their underlying mechanisms of developmental neurotoxicity, in this case involving glutamate. The dichotomy between the effects of chlorpyrifos and diazinon on glutamate transporter genes and on the cystine/glutamate exchanger are likely to play an important role in the greater susceptibility of developing neurons to chlorpyrifos-induced excitotoxicity [44,54], and ultimately in the different patterns of synaptic damage and behavioral deficits seen with each agent [39,43,45–48,50,51,56,61,65]. At the same time, we found surprising concordance in the effects of diazinon with unrelated neurotoxicants, dieldrin and Ni^{2+}, indicating these dissimilar compounds nonetheless converge on common final pathways of neurotoxicity. These results with an in vitro system can thus guide future in vivo studies to evaluate the role of excitatory mechanisms in the developmental neurotoxicity of organophosphates and other toxicants and to design appropriate treatments that may protect the developing brain from injury.

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Footnotes

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