Differential Gene Expression in Normal Human Mammary Epithelial Cells Treated with Malathion Monitored by DNA Microarrays

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Organophosphate pesticides are a major source of occupational exposure in the United States. Moreover, malathion has been sprayed over major urban populations in an effort to control mosquitoes carrying West Nile virus. Previous research, reviewed by the U.S. Environmental Protection Agency, on the genotoxicity and carcinogenicity of malathion has been inconclusive, although malathion is a known endocrine disruptor. Here, interindividual variations and commonality of gene expression signatures have been studied in normal human mammary epithelial cells from four women undergoing reduction mammoplasty. The cell strains were obtained from the discarded tissues through the Cooperative Human Tissue Network (sponsors: National Cancer Institute and National Disease Research Interchange). Interindividual variation of gene expression patterns in response to malathion was observed in various clustering patterns for the four cell strains. Further clustering identified three genes with increased expression after treatment in all four cell strains. These genes were two aldo–keto reductases (*AKR1C1* and *AKR1C2*) and an estrogen-responsive gene (*EBBP*). Decreased expression of six RNA species was seen at various time points in all cell strains analyzed: plasminogen activator (*PLAT*), centromere protein F (*CPF*), replication factor C (*RFC3*), thymidylate synthetase (*TYMS*), a putative mitotic checkpoint kinase (*BUB1*), and a gene of unknown function (GenBank accession no. AI859865). Expression changes in all these genes, detected by DNA microarrays, have been verified by real-time polymerase chain reaction. Differential changes in expression of these genes may yield biomarkers that provide insight into interindividual variation in malathion toxicity. *Key words:* DNA microarray, gene expression, malathion, pesticide, toxicology. *Environ Health Perspect* 113:1046–1051 (2005). doi:10.1289/ehp.7311 available via *http://dx.doi.org/* [Online 10 May 2005]

More than half the insecticides used today in the United States are organophosphate compounds. These pesticides are preferred because of their low toxicity and low cost of manufacture. Multiple uses of these pesticides have led to an increase in environmental and occupational exposures, especially in manufacturing, agriculture, greenhouse/nursery employees, veterinarians, groomers, and teachers [Agency for Toxic Substances and Disease Registry (ATSDR) 2001]. Environmental exposures in many cases result from the community control of mosquito populations in large cities such as New York, New York, and Los Angeles, California, in an effort to decrease the incidence of diseases such as West Nile virus [New York City Department of Health (NYCDH) 1999; Windham et al. 1998]. Toxicity of all organophosphate pesticides occurs through the inhibition of acetylcholinesterase by phosphorylation, resulting in an accumulation of acetylcholine (Bolognesi 2003). This accumulation leads to pesticide illness, which can be avoided if treatment is given soon after pesticide exposure (Saxena et al. 1997). Pesticide exposure symptoms are similar to those of common illnesses such as influenza or upper respiratory infection, making pesticide illness difficult to diagnose in most cases. Analysis of gene expression alterations after common illnesses is limited. Early diagnosis of pesticide illness could be aided with the discovery of biomarkers of exposure to organophosphate pesticides.

Biomarkers of exposure for specific pesticides or pesticide classes can be determined in part with gene expression profiling. Gene expression signatures for pesticides with unknown side effects have recently been suggested as a means of defining pesticide action and discovering pesticide alternatives (Duke et al. 2003). These signatures would allow a comparison between pesticides and known carcinogens or genotoxic agents to assist in conclusively determining the effect of organophosphate pesticide exposure in human populations.

This study examines the gene expression profile of malathion, a widely used organophosphate pesticide with a potential for a high degree of environmental and occupational exposure in humans. *In vivo* animal studies of malathion exposure have shown positive results of chromosomal damage [U.S. Environmental Protection Agency (U.S. EPA) 2000a], although previous research on malathion to show genotoxicity and/or carcinogenicity has been inconclusive in humans (Windham et al. 1998). Most of these studies are generally performed on pesticide applicators, making it difficult to analyze exposure to just one pesticide (Titenko-Holland et al. 1997); however, Blasiak et al. (1999) analyzed the effect of malathion exposure alone on human peripheral blood lymphocytes. Their *in vitro* analysis indicated that malathion's two main metabolites, malaoxon and isomalathion, did in fact act as genotoxic compounds after only 1 hr of exposure, whereas pure-grade malathion had no such effect. These two metabolites have been found as contaminants in the technical-grade malathion commonly used today. To confirm and extend these results, further *in vitro* studies are needed.

The present study, using malathion with 98.6% purity, was designed to analyze this pesticide's biologic activity at the gene expression level. The concentration used is equivalent to 0.2 mg/0.1 m^3, which is 10-fold less than the no observed adverse effect level for acute exposures in humans at which early signs of malathion-related illness have been reported (ATSDR 2001). Gene expression was measured by microarrays after exposure to malathion for both 6 and 24 hr, allowing more time for metabolite formation in the model system used. The model system was chosen partly for its ease of access to normal tissues as well as its ease of use. This system, normal human mammary epithelial cells (NHMECs), was selected not only for analysis of gene expression signatures after malathion exposure but also to assess interindividual variation in response to this pesticide. Although the use of immortalized cell lines related to neurologic and/or respiratory response may in some ways seem a more viable model, these types of cell lines would not yield any information about interindividual variation of response. Variable response in human cell strains may lead to the discovery of candidate biomarkers related to at-risk worker populations, whereas the gene expression profile generally gives the potential to support genotoxicity and carcinogenicity of malathion.

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Further research focusing on the response of any biomarkers discovered in this model could then be performed in cell lines related to clinical end points of malathion exposure.

Materials and Methods

Cell culture. Primary NHMECs were derived from tissues salvaged at reduction mammoplasty and obtained through the Cooperative Human Tissue Network (National Cancer Institute and National Disease Research Interchange, Bethesda, MD). Development and characterization of cell strains were achieved using standard methods (Stampfer 1985). Cells were grown in mammary epithelium growth media (MEGM; Clonetics, Cambrex, Pittsburgh, PA) at 37°C and 5% CO_2.

Malathion treatment. Treatment was performed on cells in passage six that were 70% confluent. The malathion used was 98.6% pure, based on chemical analysis from the company (Sigma-Aldrich, St. Louis, MO). Preliminary studies analyzed a range of malathion concentrations (25–100 µg/mL) and time points (0–24 hr) and showed minimal toxicity at 24 hr with a final concentration of 50 µg/mL. Cells were treated by diluting the stock malathion/dimethyl sulfoxide (DMSO) mixture in media and adding this solution to aspirated cells, allowing even exposure to all cells. DMSO (0.001%) alone was used as a vehicle control. All treatments were performed in triplicate. At the end of the treatment period, cells were removed for RNA isolation. Cell viability was determined by trypan blue exclusion assay.

Microarray analysis. Microarray analysis was performed in triplicate using U133A high-density oligonucleotide microarrays (Affymetrix, Santa Clara, CA). Protocols were followed from Affymetrix *GeneChip Expression Analysis Technical Manual* (Affymetrix, 2001). Briefly, RNA was isolated from cells with Trizol (Gibco, Grand Island, NY) followed by purification with RNEasy Mini Kit (Qiagen, Valencia, CA). Spectrophotometer measurements were required to give a 260/280 ratio of 1.9–2.1 for use in microarray analysis. Double-stranded cDNA was then synthesized from total RNA (Superscript Choice System; Invitrogen, Carlsbad, CA). An *in vitro* transcription reaction (Enzo, Farmingdale, NY) was then performed to produce biotin-labeled cRNA from the cDNA. Excess biotinylated dUTPs were removed by RNEasy Mini Kit before being fragmented and added to a hybridization cocktail including eukaryotic hybridization controls (Affymetrix), bovine serum albumin and herring sperm DNA (Gibco) and biotinylated anti-streptavidin antibody (Vector Laboratories, Burlingame, CA). Hybridization on microarrays was performed for 16 hr at 45°C in a gene chip hybridization oven with rocker (Affymetrix). Microarrays were washed and stained using the protocol as described in the Affymetrix *GeneChip Expression Analysis Technical Manual* (Affymetrix, 2001) with the GeneChip Fluidics Station 400 (Affymetrix). Arrays were then scanned with an Affymetrix scanner (Hewlett Packard, Palo Alto, CA). Expression profiles were analyzed using Microarray Suite 5.0, MicroDB 3.0, and Data Mining Tool 3.0 (Affymetrix). Affymetrix arrays are produced using multiple 25-mer oligonucleotides (11–20 per target gene). Each oligonucleotide is created to match the selected region of the target gene (perfect match, PM), and a similar oligonucleotide is created altered in the 13th position to control for nonspecific binding (mismatch, MM). Results are given in signal intensities with a *p*-value determined from perfect match/mismatch (PM/MM) intensities by Tukey's biweight analysis. The average intensity values obtained for each treatment time point were compared with those of the control treatment (DMSO) to determine the effect of the malathion exposure. Signal log ratio (SLR) was determined by comparison of the signal intensities for the baseline (vehicle control) and the treatment array. An SLR of 0 represents no change in gene expression as a result of malathion exposure. An SLR of 1 is equivalent to a 2-fold change between the treatment and control. The results described here are representative of triplicates, with an average percent variability between duplicate arrays of 1.5%. The average of intensity values obtained for each treatment time point was compared with that of the control treatment (DMSO) to determine the effect of the malathion exposure. Significance of comparison was determined by *t*-test values of $p \leq 0.05$. Only relative changes ≥ 0.6 SLR were considered a significant change as a result of exposure. Gene chip analysis was performed by self-organizing map (SOM) clustering, focusing on genes with a detection *p*-value of ≤0.05 at one or more time points.

Real-time polymerase chain reaction (RT-PCR) analysis. cDNA synthesized from each sample as in the Affymetrix analysis (Invitrogen) was used in a one-step real-time polymerase chain reaction (RT-PCR) analysis reaction. Analysis was performed in duplicate on the ABI 7700 cycler, with the SYBR Green Master Mix (ABI; Applied Biosystems, Foster City, CA). Primers were designed using Primer Express (ABI) to yield unique fragments for each gene under study. Reactions were set up following recommended protocols using 100 pmol of each primer (Sigma-Genosys, The Woodlands, TX) and approximately 60 ng of template per reaction. Reactions were performed in duplicate for each sample for 40 cycles (95°C/15 sec denaturing step; 60°C/1 min annealing/extension step). Fold change was determined based on average cycle threshold (C_T) values for all duplicates and converted to SLR.

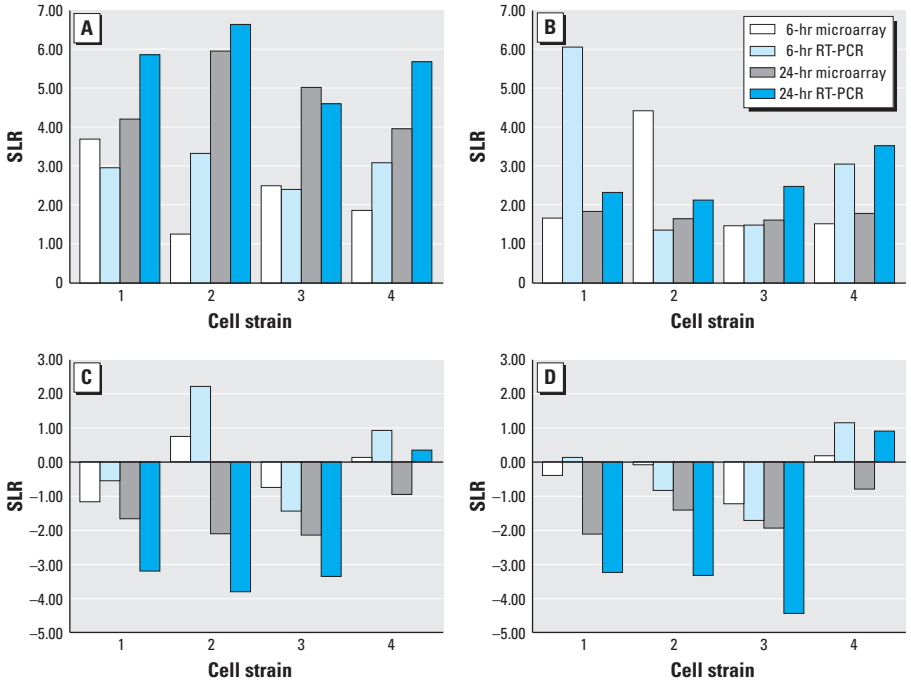

Figure 1. Gene expression patterns for four representative genes after exposure to malathion for 6 and 24 hr for both microarray and RT-PCR analysis: (*A*) aldo–keto reductase 1C1; (*B*) estrogen-responsive b-box protein; (*C*) thymidylate synthetase; (*D*) replication factor C. Results for all are shown as cell strain versus SLR for each of four cell strains. Results are from comparison of averaged signal intensities for both treated and control.

Table 1. Genes altered after exposure to malathion (SLR).[a]

Gene symbol (accession no.)[b]	6-hr microarray SLR	6-hr microarray *p*-Value	6-hr RT-PCR	24-hr microarray SLR	24-hr microarray *p*-Value	24-hr RT-PCR
AKR1C1 (M86609)						
1	3.69	0.30	2.94	4.22	0.14	5.86
2	1.23	0.07	3.32	5.93	0.00*	6.60
3	2.47	0.00*	2.35	5.01	0.01*	4.58
4	1.82	0.01*	3.05	3.92	0.00*	5.65
AKR1C2 (U05861)						
1	3.55	0.31	4.13	4.18	0.14	4.78
2	1.92	0.01*	–0.54	6.08	0.01*	0.24
3	3.09	0.00*	1.88	5.56	0.00*	4.12
4	1.39	0.08	1.33	3.36	0.00*	3.63
EBBP (NM_006470)						
1	1.69	0.01*	6.07	1.85	0.03*	2.33
2	4.44	0.01*	1.36	1.65	0.00*	2.12
3	1.46	0.06	1.48	1.61	0.03*	2.47
4	1.51	0.14	3.05	1.76	0.02*	3.50
Chlordecone reductase (NM_001818)						
1	3.42	0.33	0.80	3.96	0.15	3.06
2	0.96	0.03*	3.66	5.74	0.04*	3.74
3	1.82	0.01*	2.03	4.81	0.00*	4.69
4	1.71	0.00*	2.56	3.57	0.02*	4.76
BUB1 (NM_001211)						
1	–1.36	0.23	0.10	–1.91	0.17	1.25
2	0.96	0.06	1.48	–2.19	0.08	–3.47
3	–1.99	0.02*	–1.53	–1.90	0.03*	–1.89
4	0.14	0.19	–0.09	–0.75	0.01*	–0.54
TYMS (NM_001071)						
1	–1.15	0.03*	–0.54	–1.66	0.01*	–3.19
2	0.74	0.36	2.19	–2.14	0.04*	–3.84
3	–0.76	0.09	–1.49	–2.17	0.00*	–3.40
4	0.11	0.21	0.90	–0.99	0.00*	0.31
PLAT (NM_000930)						
1	–2.06	0.00*	2.57	–2.25	0.00*	–0.56
2	0.23	0.81	–1.15	–1.53	0.20	0.87
3	–2.09	0.00*	–1.50	–1.96	0.00*	–2.77
4	–0.74	0.04*	–0.94	–1.34	0.01*	–0.69
RFC3 (BC000149)						
1	–0.39	0.21	0.13	–2.11	0.02*	–3.24
2	–0.06	0.25	–0.84	–1.41	0.65	–3.32
3	–1.23	0.01*	–1.72	–1.93	0.01*	–4.41
4	0.18	0.48	1.14	–0.80	0.01*	0.89
Unknown (AI859865)						
1	0.02	0.65	NA	–2.97	0.00*	NA
2	–1.26	0.05*	NA	–1.61	0.04*	NA
3	–1.29	0.07	–2.23	–1.72	0.05*	–1.86
4	0.33	0.20	1.30	–1.11	0.05*	2.67
CDC20 (NM_001255)						
1	–4.14	0.00*	0.55	–2.57	0.00*	–2.64
2	0.61	0.01*	0.31	–1.94	0.00*	–4.06
3	–2.05	0.01*	–2.25	–3.50	0.01*	–5.06
4	0.12	0.56	–0.51	–0.61	0.04*	–0.79
CYCLINA2 (NM_001237)						
1	–0.48	0.06	0.81	–1.70	0.00*	–0.84
2	0.41	0.39	–0.97	–1.51	0.62	–5.06
3	–1.12	0.06	–0.89	–3.72	0.02*	–5.64
4	–0.03	0.50	0.30	–2.50	0.01*	1.94
CPF (NM_016343)						
1	–3.39	0.06	4.12	–2.11	0.07	0.96
2	–0.45	0.60	0.41	–2.12	0.17	–5.64
3	–2.03	0.03*	0.21	–3.88	0.02*	–5.64
4	0.41	0.00*	0.14	–1.58	0.00*	–0.84
p16 (AF115544)						
1	–1.88	0.50	1.79	–2.53	0.03*	0.77
2	–1.18	0.02*	–2.06	–1.55	0.17	–2.40
3	–2.51	0.17	0.11	–4.15	0.05*	–1.47
4	0.50	0.18	0.29	–0.12	0.15	0.06

NA, not applicable.

[a]Data are gene expression patterns for select genes after malathion exposure for both 6 and 24 hr for both microarray and RT-PCR analysis. Genes are listed for each cell strain (1–4) with columns showing SLR for microarray analysis with the associated *p*-value along with the RT-PCR result for each time point. Statistical analysis was performed by *t*-test using signal intensity values for triplicate analyses with Data Mining Tool 3.0 (Affymetrix). [b]From GenBank (http://www.ncbi.nlm.nih.gov/GenBank). *$p \leq 0.05$.

Results

Trypan blue exclusion test. Trypan blue was used to analyze toxicity by measuring cell viability for each cell strain for each treatment. The results showed a range of viability of 92–97% at all time points for all strains analyzed (results not shown).

DNA microarray. Microarray analysis focused on genes altered by 1.5-fold change (±0.6 SLR) with a p-value ≤ 0.05, which yielded varying numbers of genes altered for each cell strain (strain 1: 674 increased, 408 decreased; strain 2: 382 increased, 411 decreased; strain 3: 1,058 increased, 1,019 decreased; strain 4: 714 increased, 665 decreased). The ±0.6 SLR was selected arbitrarily to include most statistically significant gene expression changes. Comparisons between cell strains at all time points yielded a large number of genes (13,712) that were used as a base for further analysis by clustering. SOM clustering for all cell strains found only three genes increased in four cell strains, and only six were found to be decreased. The three genes increased in all cell strains included two aldo–keto reductases [2α-hydroxysteroid dehydrogenase (*AKR1C1*) and 3α-hydroxysteroid dehydrogenase (*AKR1C2*)] and an estrogen-responsive gene (*EBBP*). The genes decreased included plasminogen activator (*PLAT*), centromere protein F (*CPF*), replication factor C (*RFC3*), thymidylate synthetase (*TYMS*), putative mitotic checkpoint kinase (*BUB1*), and a gene of unknown function (GenBank accession no. AI859865; http://www.ncbi.nlm.nih.gov/GenBank). Four examples of these are shown in Figure 1. Further SOM analysis looked at genes altered in three of the four cell strains analyzed, including another aldo–keto reductase, pseudo-chlordecone reductase (*AKR1C4*). Those decreased in three of four cell strains included cyclinA2 (*CCNA2*), cyclin-dependent kinase inhibitor (*p16INK4A*), and cell division control 20 (*CDC20*) (Table 1). The full list of genes altered in each cell strain can be found in the publication by Gwinn et al. (2004b), including genes found to be altered in at least two of four cell strains analyzed. Venn diagrams show the breakdown of these gene expression patterns (Figure 2A,B). Examples from this list are *Hsp40* (GenBank accession no. BC095400) and stress-associated endoplasmic reticulum protein (GenBank accession no. BF747267), both involved in stress response and increased after malathion exposure. Cytochrome b_5 reductase (GenBank accession no. AF169802) is also increased after exposure to malathion, although this gene is involved in xenobiotic metabolism similar to the aldo–keto reductase gene family members increased in all cell strains analyzed. Those decreased in at least two cell strains include genes involved in protein modification and

cyclin degradation [ubiquitin carrier protein E2C (GenBank accession no. NM_00799) and ubiquitin-conjugating enzyme E2-EPF (GenBank accession no. NG_001583), respectively] along with genes for structural proteins α- and β-tubulin (GenBank accession nos. AF141347, AI860746). These genes along with others found on this list, because they are altered in only two of four cell strains analyzed, represent the interindividual variation found in response to malathion exposure.

RT-PCR. RT-PCR results confirmed those found by microarray analysis for genes analyzed, with a Pearson's correlation analysis r^2 of 0.70 ($p \leq 0.0001$). Analysis was performed for select genes as shown in Table 1, including genes in Figure 1 showing the comparison between microarray and RT-PCR results. Some variability can be seen for some time points, but the direction of change remains constant. Variable results include those results found to have no significant change by microarray analysis as measured by *t*-test. Also, RT-PCR showed increased levels of change in many cases (*TYMS*, *EBBP*, *RFC3*) most likely related to the increased specificity of RT-PCR. Microarray results unconfirmed by RT-PCR are most likely related to differences in sequences used in primer design compared with those used in probe design on the microarray. Despite these variations, these genes were included for discussion purposes and for inclusion in future studies.

Discussion

In the United States today, organophosphates make up the major class of pesticides (U.S. EPA 2003b), yet little is known about their potential genomic effects. The goal of the present study was to compile information on changes in gene expression profiles after exposure to the pesticide malathion. Previous research to determine the biologic effects of malathion has been inconclusive (U.S. EPA 2003a). To learn more about malathion's toxicity, microarray analysis was used to analyze the expression profile of malathion in NHMECs. NHMECs were selected as the model system because of the availability of normal tissue, which allows analysis of both the general effects of exposure and those related to interindividual differences.

Similar gene expression alterations in response to malathion exposure were found in all cell strains for nine genes: *a*) aldo–keto reductase 1 (*AKR1C1*), *b*) aldo–keto reductase 2 (*AKR1C2*), *c*) an estrogen-responsive gene (*EBBP*), *d*) plasminogen activator (*PLAT*), *e*) centromere protein F (*CPF*), *f*) replication factor C (*RFC3*), *g*) thymidylate synthetase (*TYMS*), *h*) putative mitotic checkpoint kinase (*BUB1*), and *i*) a gene of unknown function (AI859865). Of these, those increased (*a–c*) are potentially involved in carcinogen and steroid metabolism, whereas some of those decreased are associated with DNA replication (*e–g*) and cell cycle progression (*h*). The aldo–keto reductase gene family members are involved in the breakdown and eradication of endogenous and exogenous substrates, including steroids and pesticides. Recent studies have implicated these genes in the activation of polycyclic aromatic hydrocarbons, suggesting a potentiation of carcinogenicity in the presence of mixed exposures (Palackal et al. 2002; Penning et al. 1996). Three of the six genes found to be decreased after malathion exposure are involved in DNA replication, with altered expression resulting in cell cycle arrest (Ellison and Stillman 2003; Testa et al. 1994; Trinh et al. 2002). The similarities in the known biologic functions of these genes and their relationship to carcinogenesis as shown by current literature suggest that exposure to malathion may increase the possibility of carcinogenesis. Regardless, these nine genes are a starting point in the search for a genetic biomarker of exposure to malathion, and perhaps other pesticides. Of the three genes found to be increased, *AKR1C1* and *AKR1C2* were also increased after exposure to a non-organophosphate pesticide, oxythioquinox (Gwinn et al. 2004a). Therefore, these two genes may be good general markers of pesticide exposure, with perhaps *EBBP* as a specific marker for malathion exposure. Follow-up studies need to be performed on these nine genes, not only in an increased number of cell strains (*in vitro*) but also in an exposed worker population to confirm expression patterns *in vivo*. Further analysis of an increased number of normal human cell strains to support these results will show whether these nine genes are consistently altered regardless of interindividual variation. Results from these studies could be used to select one or two genes as markers in an epidemiology study with exposed workers. Ideally, workers exposed only to malathion could then be compared with those exposed to a mixture of pesticides, but this would depend on population availability.

Highlighting the importance of interindividual variation, three of the four cell strains showed similar alterations in select genes. Those genes increased in three of the four strains include an additional member of the aldo–keto reductase family, pseudo-chlordecone reductase. Like those listed above, this gene is involved in steroid metabolism, as well as potential carcinogen metabolism (Jez et al.

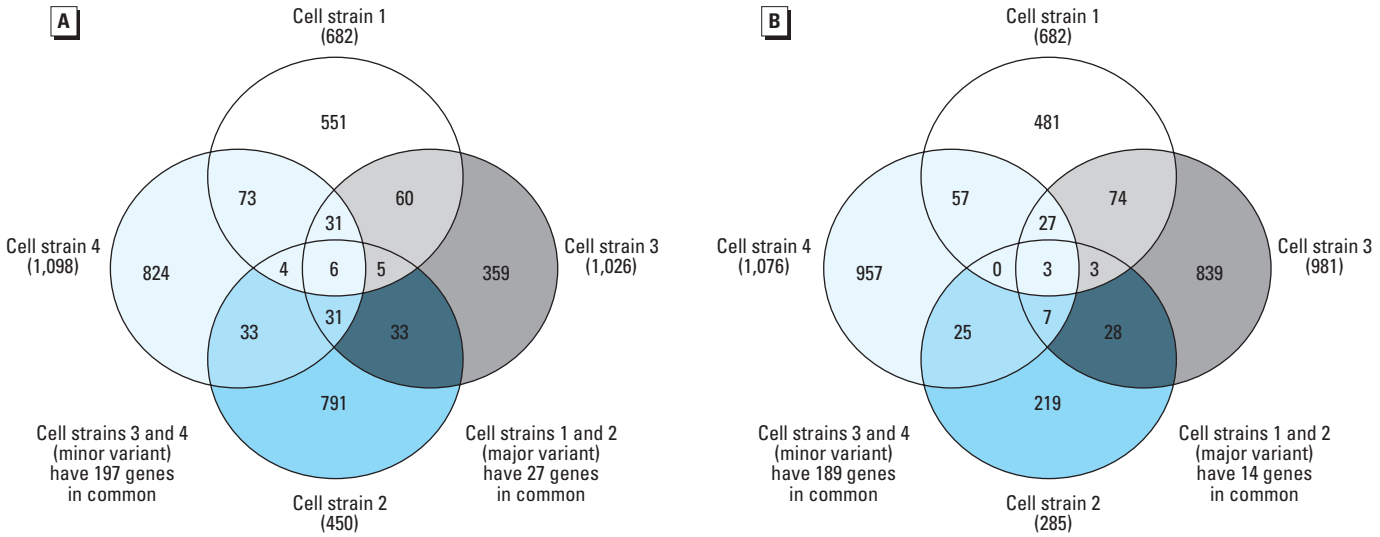

Figure 2. Gene expression patterns for all four cell strains shown as Venn diagrams illustrating the interaction in gene numbers for each of four cell strains. (*A*) Genes increased in each cell strain and (*B*) genes decreased in each cell strain are shown. Numbers of genes altered similarly for the major and minor variants are given outside the diagrams, and numbers of remaining genes are shown within the diagrams. Using genes that were present on at least one array, only six genes were found to be increased in all four cell strains, and only three genes were found to be decreased in all four cell strains.

1997; Kawamata et al. 2003). Genes decreased in three of the four cell strains included three genes involved in the regulation and progression of the cell cycle, *CDC20*, cyclinA2, and *p16INK4a*. *CDC20* and cyclinA2 are both involved in the cell's progression from metaphase to anaphase, with *CDC20* being necessary to activate the anaphase promoting complex leading to the degradation of cyclinA2 (Dunican et al. 2002; Yih and Lee 2003). A decrease in expression of *CDC20* would be expected to lead to an increase in cyclinA2; however, these cell strains also showed a decrease of cyclinA2. Whether this decrease is a result of a feedback inhibition or a result of malathion's mechanism is not known. The third gene, *p16INK4a*, is also involved in preventing anaphase promotion by disrupting cyclin D/CDK4 kinase complex. Inactivation of *p16* along with *p14* has been associated with squamous cell carcinoma (Smeds et al. 2002; van den Boom et al. 2003). Confirmation not only of these expression pattern alterations but also of cell cycle alterations as a result of these alterations would further suggest the role of malathion exposure in carcinogenesis. Although no studies have been published to date on chromosomal variations related to malathion exposure, some gene expression changes after exposure to malathion in this study are similar to those found in other cell culture models of chromosomal changes (Geigl et al. 2004; Waisfisz et al. 2002). These include changes in centromere protein F (GenBank accession no. NM_016343), replication factor C (GenBank accession no. BC000149), and *p16INK4A* (GenBank accession no. AF115544). Decreases in expression of these three genes were found in all four cell strains and may suggest an increase in chromosome instability.

Interindividual variation as a result of genetic polymorphisms in genes of interest would focus on specific at-risk worker populations. For example, the four cell strains analyzed in this study have been genotyped for a variety of genes, particularly those involved in cell cycle control and xenobiotic metabolism. Two of the four strains selected for analysis are heterozygous for an intermediate variant haplotype of *p53*, a cell cycle control gene. Molecular epidemiologic studies have implicated this haplotype of three *p53* polymorphisms [the codon 72 amino acid substitution (R/P); an intron 3, 16-bp insertion/deletion polymorphism; and an intron 6, single nucleotide polymorphism (A/G)] in breast cancer (Keshava et al. 2002; Sjalander et al. 1996; Wang-Gohrke et al. 1999; Weston et al. 1997) and other cancer types (Birgander et al. 1995; Pfeifer et al. 2002; Robles et al. 2002; Wu et al. 2002). Although no biologic mechanism for the role of this intermediate variant in carcinogenesis has been defined, several studies associating this haplotype with cancer support such a role. Analysis of genes altered only in the strains expressing this variant indicated three genes involved in cell cycle control, p53-inducible protein (GenBank accession no. Hs.50649), cyclin D1 (GenBank accession no. Hs.82932), and proliferating cell nuclear antigen (GenBank accession no. NM_002592), which may further support an association between malathion exposure, *p53* variant status, and carcinogenesis (Flatt et al. 2000; Musgrove et al. 1994; Yao et al. 2002). Further analysis of these genes in an increased number of cell strains with known *p53* haplotypes to confirm their expression pattern after malathion exposure is needed to confirm these results. However, given that this haplotype is found only in about 10% of the population, this varied pattern of expression in key genes in cell cycle control may highlight a specific at-risk population.

Searches for similar natural compounds to replace these potentially disruptive chemicals can also use gene expression profiles (Duke et al. 2003). Profile comparisons with that of a natural pesticide may decrease the need for organophosphates. Comparison of malathion's gene expression profile with that of well-defined chemicals such as benzo[*a*]pyrene will yield important information about malathion's role in both genotoxicity and carcinogenicity. Results of this experiment showed some similar expression patterns to those in the same cell strains exposed to benzo[*a*]pyrene (Keshava et al. 2005). Although similarities were found in expression patterns of a few genes, these did not include key genes such as the cytochrome P450s. A more focused comparison between the two expression profiles is needed to further define similarities and differences between the two chemicals. Given the neurotoxicity of organophosphates, comparisons of the response to malathion and known neurotoxins would also be of interest using expression profiles from these same cell strains.

Although people are commonly exposed to pesticides, human exposure is not well monitored (Bolognesi 2003; ATSDR 2001). Discovery of genes altered after exposure to malathion may aid in future epidemiology studies on pesticide exposures. Gene expression profiling can be used to yield genetic biomarkers of exposure that, after validation, could be used in a clinical setting for early determination of organophosphate exposure, increasing early treatment of pesticide illness and thereby increasing the recovery rate of exposed individuals.

Gene expression profiling in response to toxic chemicals can be used to seek evidence for a chemical's potential toxicity and carcinogenicity, absent testing in animals. Further, discovery of genes altered after exposure to pesticides and other chemicals may provide useful biomarkers for future studies in environmental and occupational epidemiology.

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