KEY METABOLIC PATHWAY CHANGES IN HUMAN EMBRYONIC STEM CELLS EXPOSED TO METHYL PARATHION AND METHYL PARAOXON

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ABSTRACT

Toxic industrial chemicals (TICs) represent a threat to soldiers, first responders and other civilians. One class of toxic industrial chemicals, pesticides, is particularly accessible and used widely in crop, industrial, and home applications. For many pesticides, including methyl parathion (MP), there is incomplete and sometimes conflicting information regarding the basic molecular toxicological consequences of exposure in humans. Most documented effects reported are from epidemiological studies in adult humans and laboratory studies in adult animals. It is important to consider that many chemicals, including pesticides, have dramatically different toxic effects in developing embryos. Thus, any thorough chemical or drug toxicological evaluation must examine the compound's effect on early development. Since not all cell types contain fully active metabolic enzymes required to carry out Phase I and II transformation reactions, it is important to examine the effects of both the parent compound and the active metabolite(s) normally transformed by the liver. In this work, we have compared the effects of MP and its active metabolite methyl paraoxon (MPO) on the secreted metabolic products (measured via LC-ESI-QTOF MS) found in the spent cell culture medium from MP-exposed, MPO-exposed, and control pluripotent WA09 human embryonic stem cells. Employing Stemina's devTOX teratogenicity prediction model, MPO was predicted to be tetratogenic at all 3 concentrations tested (180 µM, 130 µM, 72 µM) and MP was predicted to be tetratogenic at 500 µM, the highest concentration tested. Several hundred statistically significant differences were observed between the treated and the untreated cells with 13 human metabolic pathways exhibiting statistically significant enrichment in the treated cells. These data suggest that MP and MPO exposure may significantly impact the metabolism of undifferentiated hES cells.

METHODS

•WA09 hES cells were grown in mTeSR1® medium and exposed to concentrations equivalent to an EC_1, EC_{10} and EC_{30} for MP (1uM, 50uM, 500uM) or MPO (72uM, 130uM, 180uM). DMSO was used as the solvent.

•The metabolic profile of the secretome of the hES cells was carried out using LC-ESI-Q-TOF MS-based metabolomics. Proprietary sample enrichment and separation methods were developed by Stemina Biomarker Discovery.

•After the extracted ion chromatogram (EIC) review and putative annotation of features, raw LC-MS data files were analyzed using the "Find by Formula" routine in the Agilent MassHunter software. This semi-automated analysis is a good tool to determine if the mass spectra for a feature (the specific molecular formula for the annotation) is a reasonable match with the formula. The algorithm takes into account several factors, including ppm mass error, adducts and isotopic peak abundances, isotope

RESULTS

Table 1. Prediction of teratogenicity in MP- and MPO- exposed WA09 cells.

Treatment	Dose (uM)	Replication	Effect	Prediction	% Non	% Ter	Confidence
Methyl Parathion	1	1	Unknown	I/Ter*	0.46	0.54	.08*
Methyl Parathion	1	2	Unknown	Non	0.57	0.43	0.14
Methyl Parathion	50	1	Unknown	I/Ter*	0.47	0.53	.06*
Methyl Parathion	50	2	Unknown	I/Non*	0.54	0.46	.08*
Methyl Parathion	500	1	Unknown	Ter	0.38	**0.62**	0.24
Methyl Parathion	500	2	Unknown	Ter	0.22	**0.78**	0.56
Methyl Paraoxon	180	1	Unknown	Ter	0.18	**0.82**	0.64
Methyl Paraoxon	130	1	Unknown	Ter	0.24	**0.76**	0.52
Methyl Paraoxon	72	1	Unknown	Ter	0.28	**0.72**	0.44
Controls	**Dose (uM)**	**Replication**	**Effect**	**Prediction**	**% Non**	**% Ter**	**Confidence**
Valproate	1000	1	Ter	Ter	0.22	**0.78**	0.56
Penicillin	48	1	Non	Non	0.74	0.26	0.48
DMSO	0.10%	1	Non	Non	0.74	0.26	0.48

Table 1 Legend. Stemina's devTOX model was applied to the data from the MP- and MPO-exposed WA09 hES cells**.** Key: Ter (teratogen), Non (non-teratogen), or I (Inconclusive). Confidence values less than 0.1 are considered inconclusive and are marked with an asterisk.

Table 2. Metabolites and pathways exhibiting a statistically significant enrichment in the stem cells exposed to MPO.

Putative Metabolite	FBF Score	Pathways
(2R,4S)-2,4-Diaminopentanoate	99.2	D-Arginine and D-Ornithine metabolism
2-amino-4-oxo-pentanoic acid	81.4	D-Arginine and D-Ornithine metabolism
2-Keto-6-aminocaproate	83.9	Lys degradation
2-oxo-5-aminovalerate	81.4	Arginine and Proline metabolism
4-Acetamidobutanoic acid	83.9	Arginine and Proline metabolism
5-Amino-2-oxopentanoic acid	81.4	D-Arginine and D-Ornithine metabolism
5-Aminolevulinic acid	81.4	Gly, Ser and Thr metabolism
Alpha-ketoisovaleric acid	98.8	Val, Leu and Ile degradation
		Val, Leu and Ile biosynthesis
		Pantothenate and CoA biosynthesis
Asymmetric Dimethylarginine (ADMA)	93	Arginine and Proline metabolism
Choline	97.4	Gly, Ser and Thr metabolism
		Glycerophospholipid metabolism
cis-4-Hydroxy-D-proline	81.4	Arginine and Proline metabolism
Guanine	81.2	Purine metabolism
L-Cystathionine	71.1	Gly, Ser and Thr metabolism
		Cys and Met metabolism
L-Glutamic-gamma-semialdehyde	81.4	Arginine and Proline metabolism
L-Proline	99.7	Arginine and Proline metabolism
Malic acid	86.9	Pyruvate metabolism
		Glyoxylate/ dicarboxylate metabolism
		Citrate cycle (TCA cycle)
Ornithine	99.2	Arginine and Proline metabolism
		D-Arginine and D-Ornithine metabolism
		Glutathione metabolism
Pipecolic acid	92.5	Lys degradation
Trans-4-Hydroxy-L-Proline	81.4	Arginine and Proline metabolism
Valine	100	Val, Leu and Ile degradation
		Val, Leu and Ile biosynthesis

Table 2 Legend. Scores were assigned by Agilent's Mass Hunter program, using the "Find by Formula" (FBF) algorithm. This algorithm outputs a score (100 is perfect) for each feature/formula. Any features with a score of less than 70 were removed. To date, asymmetric dimethylarginine (ADMA), choline, L-cystathionine L-proline, ornithine (in red) have been definitely identified as significantly increased (>2 fold) in exposed cells. Other putatively identified metabolites are in the process of being definitively identified using MS-MS.

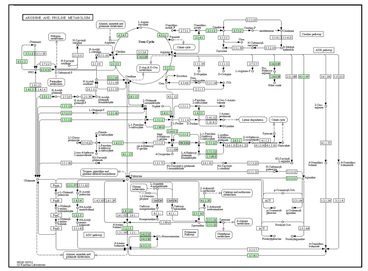

Figure 1. The arginine and proline metabolism pathway appears important because asymmetric dimethylarginine (ADMA), L-proline, ornithine, 2-oxo-5-aminovalerate, 4-acetamidobutanoic acid, cis-4-hydroxy-D-proline,L-glutamic-gamma-semialdehyde, and trans-4-hydroxy-L-proline metabolites were significantly altered in expression in the MP- and MPO-exposed cells.

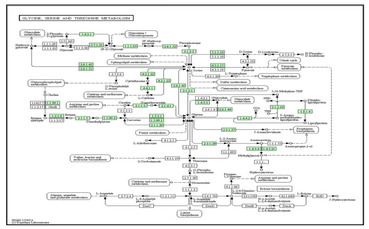

Figure 2. The glycine, serine, and threonine metabolism pathway also appears involved based on our data which showed that 5- aminolevulinic acid, choline and L-cystathionine were all significantly altered in expression in the MP and MPO exposed cells.

CONCLUSIONS

1) These data suggest that both MP and MPO significantly impact the metabolism of human embryonic stem cells.
2) Analysis using Stemina's devTox model suggests that MP (at the highest dose) and all doses tested of MPO are most likely teratogenic to a developing embryo.
3) Initial results of this study have opened a new avenue toward a better understanding of how exposure to toxic industrial chemicals may interfere with early human embryonic growth and development.

ACKNOWLEDGMENTS

This work was funded by the US Army ECBC ILIR Program. Special thanks to Ms. Kelley Betts for her technical assistance in the preparation of this poster.