Moving Ahead With Mixtures: Environmental Chemical Exposures as Risk Factors for Human Diseases and Disorders

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Outline

• Biological Continuity and Disease Complexity

• Disconnect between Experimental and Epidemiological Studies of Chemicals and Disease Complexity
  - Pesticides and Parkinson’s disease
  - Phthalates and Fungicides and male reproductive system disorders

• Approaching Mixtures: Using Disease and Disorder Endpoints to Define Relevant Mixtures
Biological Continuity and Disease Complexity

• Nature is parsimonious: molecular targets are generally well-conserved across species. Thus targets of molecules in plants, insects, etc. will often generalize to humans, hence the concerns over the role of environmental exposures in human diseases and disorders.

• It is increasingly apparent that most human diseases and disorders are complex and multi-factorial in etiology rather than the product of a single causative factor.

• The importance of non-genetic contributions, including environmental risk factors in complex disease is underscored by estimates that single gene mutations account for less than 5% of incidence, at least in the case of cancers and cardiovascular-related diseases (Willet, Science, 2002; 296:695).

• Similar considerations apply to diseases of the nervous system.
Parkinson’s Disease as a Complex Disorder

- Factors Enhancing Vulnerability
  - Age
  - Genetic background (e.g., α-synuclein, DJ1, parkin)
  - Boxing
  - Farming
  - Drinking Well Water
  - Pesticide Exposure
  - Gender
  - Diet

- Protective Factors
  - Gender
  - Smoking
  - Caffeine
  - Exercise
  - Diet
Interactions of Risk Factors Over Time Contribute to an Individual’s Health Status

- Each individual has a unique set of risk factors
- No individual is exposed to a single chemical; thus there may also be chemical interactions
In Contrast, Most Toxicology Studies Examine Toxicants as Risk Factors in Isolation

- Experimental studies: evaluate one chemical in isolation, typically in a healthy young organism
- Epidemiological studies generally focus on ‘main effects’ of an exposure, statistically controlling for any potential modifiers, e.g., gender, other chemical exposures
Multiple Chemical Exposures: At Least Two Overlapping Consequences

- **Multiple Hits and Impairment of Homeostasis**
  Exposure to an individual chemical may be insufficient to induce overt effects, whereas multiple risks, by provoking changes concurrently at multiple different target sites of the dopamine system, would impair the operation of homeostatic mechanisms, leading to dopamine dysfunction and neuronal cell death.

- **Multiple Hits Converging on a Common Adverse Outcome**
  Single insults act by different mechanisms that converge upon a common outcome resulting in cumulative toxicity; e.g., male reproductive toxicity
• Effectively examine the contribution of a chemical as a ‘sole etiology’ for an adverse outcome.
  - This assumes that the disease phenotype results from a single mechanism, which increasingly appears to be inconsistent with many diseases and disorders.
  - This assumes that the impact of a chemical exposure is so severe that it alone produces the phenotype. Under such conditions, much higher exposure levels are likely to be required than would be the case when exposure occurs in conjunction with other known risk factors.

• Consequently:

We’re picking the low-hanging fruit, i.e., the chemical contributions with the largest effects
Importance of Mixtures

• Does studying chemical exposures in the context of other pertinent risk factors, including other chemical exposures:
  – Demonstrate effects of chemical exposures at lower levels or in a synergistic or potentiated capacity?
    • outcomes with significant implications for risk assessment and public health protection
  – Provide more realistic models of the disease/disorder phenotype?
    • an outcome with implications for neuroprotective and therapeutic strategies
Parkinson’s Disease: A Disorder of the Nigrostriatal Dopamine System

• Loss of dopamine (TH+) cells in substantia nigra
• Loss of dopamine terminals and associated markers
• Reduction in levels of dopamine and metabolites and dopamine turnover
• Symptoms and signs occur with loss of approximately 80% of dopamine neurons
• Characteristic pathology: Lewy bodies, intracytoplasmic inclusions containing alpha-synuclein, ubiquitin and parkin proteins
Pesticide Exposure Link to PD

- Associations of pesticide exposure with PD in numerous epidemiological studies and case reports.
- Similarity of the herbicide paraquat to MPP⁺, the active metabolite of MPTP, a component of illegally manufactured heroin that produced severe progressive PD in young drug addicts.
Risk Factor Interactions: Pesticides and Parkinson’s Disease

Paraquat and the Parkinson’s Disease phenotype
Many Pesticides Impact Brain Dopamine Systems

- Dopaminergic Effects of the Ethylenebisdithiocarbamate Fungicide Maneb
  - Decreases locomotor activity
  - Potentiates MPTP effects on locomotor activity and catalepsy
  - Enhances the uptake of MPTP into brain
  - To date, two incidences of Parkinsonism in humans have been related to occupational maneby exposures

Will combined paraquat and maneby enhance the Parkinson’s Disease phenotype?
Pesticides Also Show Overlapping Geographical Use

Maneb
Paraquat
Mancozeb
Triadimefon

Use Based on Pounds per sq. mile
Low
High
Support for an Environmental Basis of Parkinson’s Disease

- Geographic variation in age-adjusted mortality statistics

Based on 1988 data from Lanska et al.
Young Adult Animal Model: Combined Paraquat (PQ) and Maneb (MB) and the Parkinson’s Disease Phenotype

Schedule of i.p. injections

Dose Groups:
- Saline
- 10 mg/kg paraquat
- 30 mg/kg manebed
- 10 mg/kg paraquat + 30 mg/kg manebed

6 wk male C57BL/6 mice
Combined PQ+MB Selectively Reduced Nigral TH Dopamine Neurons

Subsequently confirmed using unbiased stereology
Oxidative Stress and the PQ+MB Induced Parkinson’s Disease Phenotype

Levels of lipid peroxidation in various brain regions in the young adult model with exposures to saline, PQ alone, MB alone or combined PQ+MB
MB Increases PQ Accumulation and Delays its Clearance from Brain

What other food, chemical, drug etc might cause similar toxicokinetic interactions?
Aging and PQ+MB: Enhanced DA Cell Loss and Permanent and Progressive Effects

Measured Two Weeks and Three Months Post-Dosing
Early Development and PD Risk?

The etiological risk factors for the disease may occur at a far different time than that at which the phenotype is expressed.
Experimental Design: Postnatal Pesticide Exposure

- Can developmental insults lead to a PD phenotype?
- Can they alter vulnerability to risk factors later in life?
Decreases in DA neurons following developmental exposures are significantly enhanced when followed by an adult exposure to pesticides as compared to adult only exposures. No changes in numbers of TH- neurons.
Progressive Decline in DA Cell Loss With Age in Males Following Postnatal Exposure

Stereological counts of substantia nigra DA neurons across the lifetime following PND 5-19 exposure to saline, PQ alone, MB alone or combined PQ+MB
Sequential Administration of Paraquat & Maneb

- Can sequential environmental insults produce cumulative neurotoxicity?

**Timeline**

<table>
<thead>
<tr>
<th>GD 10 - 17</th>
<th>PN 25</th>
<th>6 WEEKS</th>
<th>7 - 8 WEEKS</th>
<th>9 WEEKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD1</td>
<td>Exposure: Saline OR 1 mg/kg MB i.p.</td>
<td>PUPS WEANED</td>
<td>LOCOMOTOR ACTIVITY</td>
<td>Re-Challenge: Saline, 5 mg/kg PQ, OR 30 mg/kg MB (every day for 8 days)</td>
</tr>
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Sequential MB Followed by PQ Later in Life Kills DA Neurons

Barlow et al., 2004
Parkinson’s Disease and Residential Exposure to Paraquat and Maneb from Agricultural Applications in the Central Valley of California

- PD risk increased for DAT A clade diplotype OR=1.66) and for 3’VNTR (OR=1.8).
- High exposure to PQ + MB increased PD risk 3 fold in carriers of one susceptibility allele (OR=2.99)
- High exposure to PQ+MB increased PD risk >4 fold in carriers of 2+ alleles (OR=4.53).
- Same effects were found for occupational pesticide exposures

Ritz et al., 2009. Am. J. Epidemiology, vol. 169:919-926
Phthalates are Anti-Androgenic

Via their ability to decrease testosterone, some specific phthalates produce a host of adverse Effects on the male Reproductive system, including hypospadias, cryptorchidism, nipple retention, reduced anogenital distance and reductions in fertility.
Consequently, Other Anti-Androgens Should be Included in Cumulative Risk Assessments

e.g., vinclozolin, procydimide, linuron, prochloraz,azole fungicides, polybrominated diphenyl ethers, dioxin, some PCBs
Cumulative Effects of In Utero Administration of Mixtures of “Antiandrogens” on Male Rat Reproductive Development

We also conducted a mixture study combining seven “antiandrogens” together. These chemicals elicit antiandrogenic effects at two different sites in the androgen signaling pathway (i.e., AR antagonist or inhibition of androgen synthesis). In this study, the complex mixture behaved in a dose-additive manner. Our results indicate that compounds that act by disparate mechanisms of toxicity display cumulative dose-additive effects when present in combination.
Mixture Effects are Not Considered in Risk Assessment

- Potentiated effects (no effect of treatment A or B alone, but significant effects when A and B are combined) will never be captured by current risk assessment methodology
  - Do 10-fold safety factors adequately encompass the risks?
- Cumulative and silent neurotoxicity are not captured in current neurotoxicology risk assessment
- How do we modify the risk paradigm to accommodate interactions?
- How do we define which interactions (mixtures) to assess?
With So Many Chemicals, Where Do We Begin?

• In the view of the NRC Committee report “Phthalates and Cumulative Risk; The Task Ahead”, cumulative risks strategies should not be specific to phthalates:
  – “…it is plausible and warranted to extend cumulative risk assessment to include chemicals associated with common adverse outcomes, as exemplified in this report by inclusion of other antiandrogenic chemicals with phthalates.”
Moving Ahead with Mixtures

- “To cite another example, EPA could evaluate combined exposures to lead, methylmercury and polychlorinated biphenyls because all contribute to the cumulative risk of cognitive deficits associated with IQ reductions in children, although the deficits are produced by different mechanisms of action.”

Such a strategy defines the relevant mixtures that should be studied based upon a defined health endpoint, an approach consistent with public health protection and with the mission of the U.S. Environmental Protection Agency.