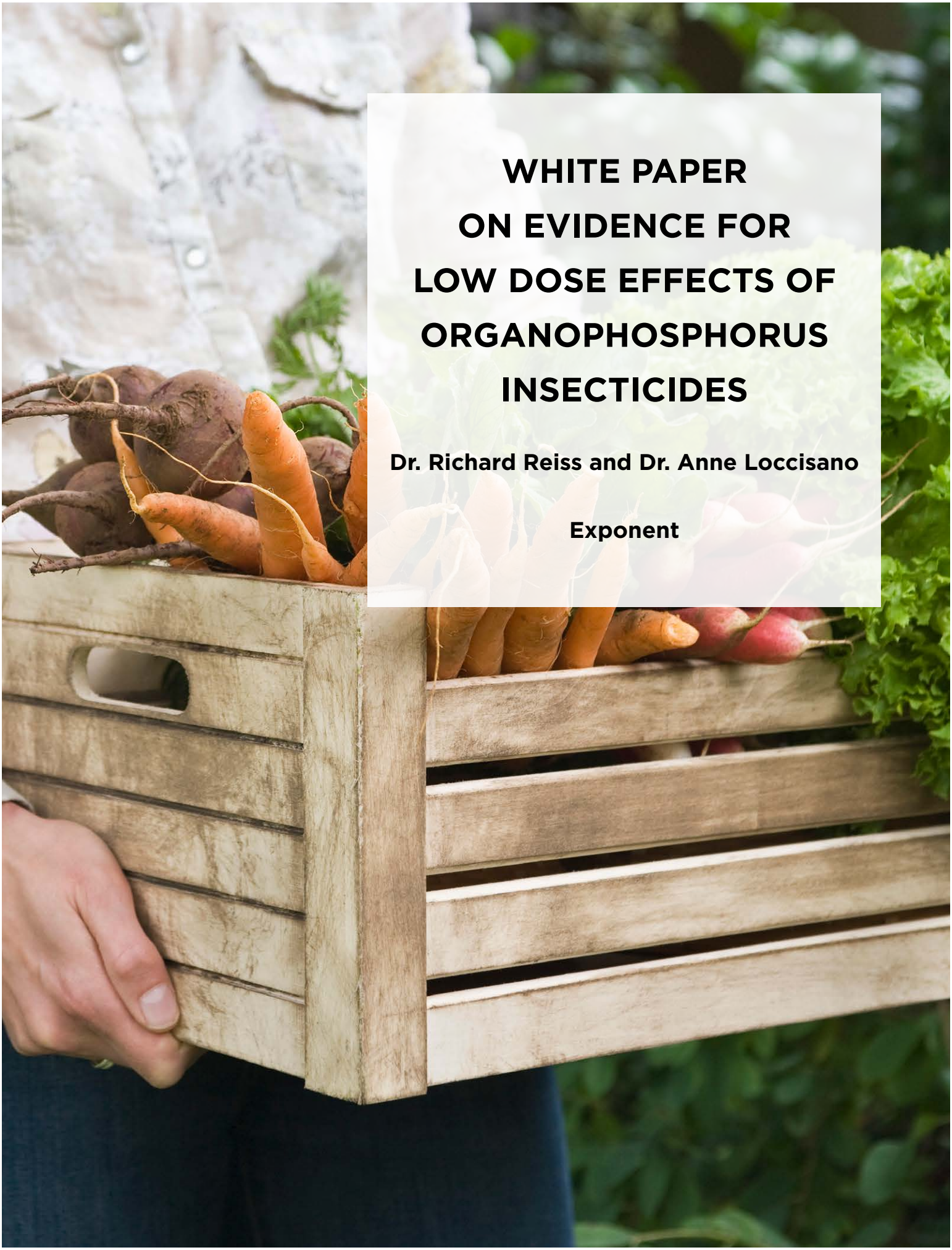

WHITE PAPER ON EVIDENCE FOR LOW DOSE EFFECTS OF ORGANOPHOSPHORUS INSECTICIDES

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Introduction

Organophosphorus insecticides (OPs) are well-studied compounds due to their widespread use in agriculture. Many studies in humans and animals have been published that assess the toxicity of this class of compounds. High doses of OPs can cause neurotoxicity. Consumers need balanced, scientifically-based, and practical information about OPs, and this white paper summarizes peer-reviewed scientific data regarding OP exposure and potential health effects, specifically, birth and neurodevelopmental effects.

Recently, a number of epidemiology (human) studies have reported associations between prenatal (maternal) or early childhood exposure to low levels OPs, and adverse birth and neurodevelopmental outcomes. These studies (the methods used, the evidence used, and the associations reported) have recently been evaluated, based on well-established, peer-reviewed science (Reiss et al. 2015). Based on these analyses, the reported associations between exposure and birth and neurodevelopmental outcomes are weak, and the measured OP levels in the studies are too low to cause a meaningful effect. The exposures reported in the studies are well below the safe exposure levels set by the United States Environmental Protection Agency (USEPA). At low-level exposures to OPs, clear evidence of adverse birth or neurodevelopmental outcomes has not been demonstrated in humans. Further data are needed in order establish any connections between low-level OP exposure and any resulting birth or neurodevelopmental effects.

What are OPs and how are humans exposed to them?

OPs are a class of compounds with wide-spectrum use in controlling insect pests on agricultural commodities (food crops, poultry, milk, eggs, meat), industrial sites, golf courses, wood products, in food-handling establishments, and also for mosquito control; most residential (home and garden) uses have been phased out. All OPs in use today are neurotoxicants, meaning that they exert their toxic effects by poisoning the nervous system of target organisms, which include insects, birds, amphibians, and mammals. OPs act on the nervous system by inhibiting an enzyme known as acetylcholinesterase (AChE), ultimately resulting in neurotoxicity. Inhibition of this enzyme is considered to be the primary toxic effect resulting from OP exposure in both animals and humans and is used by regulatory agencies to set safe exposure levels (USEPA 2000).

Exposure to OPs can occur orally, by inhalation, or by dermal contact. For the general population (which is considered residential or non-occupational exposure), oral exposure can occur by ingestion of food or drinking water containing traces of OPs or from hand-to-mouth contact with surfaces containing chemical residue. Inhalation exposure can occur as a result of spray drift from treated crop fields or golf courses or following aerial mosquitocide (spray) use. Dermal exposure can occur as a result of skin contact with treated surfaces or turf. Occupational exposure to OPs can occur by the same routes prior to or while applying insecticides. The USEPA sets tolerances for how much pesticide residue can remain on

foods, in drinking water, or on treated surfaces in order to minimize exposures, and estimated exposures for the general population are usually considered to be below regulatory limits. Regulatory limits are set sufficiently low that AChE inhibition, and thus, neurotoxicity will not occur (USEPA 2000).

While AChE inhibition is regarded as the primary effect of exposure to OPs, it is possible that developmental neurotoxicity (neurological deficits in offspring resulting from maternal exposure or early childhood exposure) may result from pathways other than AChE inhibition. Animal studies have reported some neurodevelopmental outcomes in offspring; however, high doses of chemicals are used in animal experiments (much higher than what the general population would be exposed to), and in additional to any neurodevelopmental effects, some degree of AChE inhibition has been observed (USEPA 2015). The USEPA has determined that exposure levels set to protect against AChE inhibition will also be protective of effects on the developing brain. As there are no studies with low doses (those typical of the general human population) that definitively do not inhibit AChE, there are no toxicological data in animals that indicate whether or not there would be neurotoxic outcomes at those low exposures; no biological mechanism between OP exposure and neurodevelopmental outcomes has been established.

Several human studies have been conducted over the last several years to examine whether adverse birth outcomes or developmental neurotoxicity could be detected in infants or children following maternal exposure during pregnancy or early childhood exposure to low levels of OPs. The OP levels measured in these studies are far below those needed to cause AChE inhibition based on animal and available human toxicology data (Reiss et al. 2015). The evidence from these studies has recently been evaluated, based on well-established, peer-reviewed science (Reiss et al. 2015). The findings from these analyses are summarized below, and they do not indicate that the human study data establishes that low-level exposure to OPs causes adverse birth or neurodevelopmental effects.

OPs and DAPs

OPs are metabolized by the body to other compounds, most of which are known as dialkyl phosphates (DAPs). DAPs do not exert the same effects as OPs, and they are not considered to be toxic (CDC 2013). These compounds are excreted in urine, can be measured, and are regarded as indicators of exposure to OPs. They are found in urine following exposures to very low levels of OPs that do not cause neurotoxicity. Thus, finding a measurable level of DAPs in urine does not indicate risk of an adverse health effect. In addition to metabolism of OPs by the body, DAPs are also formed in the environment and on food, so DAPs measured in urine may reflect exposure to the DAPs themselves and not exposure to an OP insecticide (Zhang et al. 2008; Chen et al. 2012). In addition, different OPs can be broken down into the same DAPs, meaning that measurement of DAPs in urine cannot be linked to exposure to a specific OP insecticide (Duggan et al. 2003; Sudakin and Stone 2011). Approximately 75% of OPs registered for use in the US break down to measurable DAPs (CDC 2013). OP insecticides also exhibit a wide range of toxicities. Large differences in toxicity and lack of specificity limit the ability of urinary DAP measurements to provide information on toxic exposures.

DAPs are excreted rapidly by the body (within 24-48 hours) and thus reflect recent exposures to DAPs, likely within the previous few days (WHO 1996). A single time point urinary measurement of DAPs does not reflect past or average daily exposure. For example, a single DAP measurement taken from a pregnant woman at one time during her pregnancy will not reflect her exposure during the entire time she was pregnant. Variability in DAP measurements is quite high, meaning that several samples taken from the same individual over the course of a few days can vary greatly in magnitude (Bradman et al. 2013) (Spaan et al. 2015). If exposure is estimated based on only one sample, this can lead over- or underestimation of actual exposure. Many of the human studies that were evaluated for associations between maternal or child OP exposure and outcomes in infants or children utilized only one or two urinary DAP

measurements to assess exposure and to make conclusions based on average or daily exposures, which limits the validity of the study results.

Epidemiology—what is it and how it is used?

Epidemiology is the study and analysis of the patterns, causes, and effects in a specified human population. Studies in this area aim to form a causal, or cause-and-effect, relationship between exposures (for example, smoking, alcohol use, or chemicals) and an adverse outcome (for example, birth defects, a disease, or death) by examining associations between the exposures and outcomes. Epidemiology studies can be conducted in different ways; in the studies that have reported associations between OP exposures and adverse birth or developmental outcomes, pregnant women or children from the general population were selected for the studies based on their exposure to OPs, and these individuals were then followed over time to assess birth outcomes in infants (such as fetal growth, birth weight or length, and head circumference) or developmental outcomes in the children (such as mental or psychological development, attention deficit hyperactivity disorder (ADHD), or behavioral problems). The evidence gathered was then used to examine associations between measured DAP levels (as an estimate of OP exposure) and any observed birth or developmental outcomes in the infants or children.

While epidemiology studies are useful for examining realistic exposures and responses in humans, there are general limitations to human studies, including the following:

- Accurate and complete assessment of exposures cannot always be performed as exposure is not controlled.
- Confounding factors that may affect health (such as diet, smoking, exposure other chemicals, or socioeconomic status) cannot always be controlled for.
- Only a limited number of responses can be studied.
- The population studied may not be representative of the general population.

In addition to the general limitations of epidemiologic studies, there are limitations specific to the studies which examined prenatal or early childhood OP exposure and birth and developmental outcomes (Raffaele et al. 2011; Reiss et al. 2015). These include:

- Limited exposure information was collected. Most studies measured urinary DAPs in the mother one or two times during pregnancy or one or two times in children, which does not reflect long term exposure or changing levels of exposure. This severely limits exposure estimation and thus the study results.
- Measurement of maternal or child DAP levels were used to estimate exposure. As discussed above, DAPs are not specific to a single OP insecticide, and DAPs are also present in the environment; OP exposure is not the only source of DAPs. Use of DAP levels cannot distinguish the exposure source, as an individual could have been exposed to an OP or simply exposed directly to DAPs.
- Confounding factors, such as diet, exposure to other chemicals, and child nutrition, were not adjusted for in all studies, which can influence the responses measured.
- There is high variability in estimated exposures across the studies, which limits how the results can be compared.

How are epidemiologic studies and results evaluated?

Epidemiologic studies seek to discover a causal relationship between exposure and an outcome by examining associations between exposures and outcomes. While these studies can identify agents that are associated with an increased risk of a disease or adverse effect, it is important to note that an association is not the same as causation. An association identified in an epidemiologic study may or may not be causal, and assessing whether the association is causal

requires an analysis of the strengths and weaknesses of the study. Epidemiological studies can provide evidence that a chemical could have caused, but not that it did cause, a particular outcome or adverse effect, and can only point out a possible connection between an exposure and outcome.

In order to evaluate the results of the human OP studies, a common and established set of guidelines, known as the Bradford-Hill criteria (Hill 1965; Gordis 2013), was applied (Reiss et al. 2015). These guidelines are used to assess the evidence for a causal relationship between an exposure and an adverse outcome; in this case, they were applied evaluate whether or not any causal relationships exist between maternal or child OP exposure and birth or neurodevelopmental outcomes in infants and children. Eleven studies with seven different populations examined associations between maternal OP exposure and birth outcomes (such as length of gestation time, birth length, birth weight, and pre-term delivery). Twenty studies with ten different populations examined associations between OP exposure and neurodevelopmental outcomes in children (such as mental and psychomotor development, IQ and memory development, attention deficit hyperactivity disorder, and behavior); these studies examined either maternal exposure to OPs or child exposure to OPs by urinary DAP measurements. The criteria used and how these criteria were not met in order establish causal relationships from these studies is discussed below.

Strength: The strength of an association is defined by the size of the reported association and is measured by appropriate statistical tests. The stronger the association, the more likely it is that the relationship is causal. For both birth outcomes in infants and neurodevelopmental outcomes in children, the associations were found to be weak. Reported associations were generally small and may not be significant. These weak associations are more likely to be explained by confounding factors or chance. For both birth and developmental outcomes, the strength of the reported associations could not be clearly and easily compared across studies due to the inconsistencies in how exposures were measured, classification methods, and how results were reported. The weak and non-significant associations are consistent with no association between DAPs and birth or neurodevelopmental outcomes.

Consistency: If the relationships reported are causal, then the association should be consistently observed when the results are replicated across different studies that used different populations and different methods. For birth outcomes, reported associations across studies were mostly inconsistent; those that were consistent were not significant. These findings do not support a causal relationship between DAP exposure and birth outcomes in infants. For neurodevelopmental outcomes in children, only a few studies were directly comparable as there were differences in the tests administered to the children, the children's age groups, and how exposure was assessed. Most of the studies showed no association with DAP exposure and child outcomes. In the few studies that could be compared, data were insufficient to establish any consistent associations between DAP exposure and neurodevelopmental outcomes in children.

Temporality: This means that the exposure has to occur before the effect is observed. Due to the limitations with use of DAP metabolites as a measure of exposure as discussed previously, assessment of the temporal relationship between DAP levels and birth outcomes was limited. Maternal DAP levels measured just prior to giving birth would be unlikely to have an influence on fetal growth over the entire gestation period. As DAPs are quickly eliminated from the body and show large variability within individuals, one or two samples would not represent past or average exposure for an individual. It is not known if exposures earlier or later during pregnancy may have an influence on fetal growth or length of gestation; thus, the DAP measurements may not be relevant as they may not have been analyzed at a time when exposure might affect the fetus. The same issues apply to neurodevelopmental outcomes; little is known regarding the time of exposure and how it affects neurodevelopment. DAP measurement prior to birth or in early

childhood does not strengthen the evidence of causal associations between birth outcomes or neurodevelopmental outcomes.

Dose-response: A dose-response relationship describes the change in observed effects caused by different exposure levels. For example, if higher exposures are observed, a higher incidence of the effect is often observed. A direct relationship between exposure and the effect observed provides support for a causal relationship; however, simply because exposure occurs or can be measured/detected, does not mean than an adverse effect will occur. For both birth and neurodevelopmental outcomes, very few significant trends were observed; the trends that were detected were weak. The lack of a dose-response relationship does not provide support for a causal relationship between OP exposure and birth or developmental outcomes. As discussed above, the primary effect of OPs is AChE inhibition, and the exposures measured in these studies are far below those levels that would result in significant AChE inhibition. Other mechanisms of toxicity for OPs have been hypothesized; however, no effects at the levels measured in the studies have been clearly established.

Plausibility and coherence: The association should agree with current understanding of biological processes and existing scientific knowledge; there has to be some logical basis for proposing an association between the exposure and the effect. For OPs/DAPs and birth and neurodevelopmental outcomes, no biological connection has been established through either animal or human studies. At high exposures, OPs can cause neurotoxicity in adults (USEPA 2000); however, the levels measured in the human studies discussed here are far lower than those that would cause neurotoxicity or even significant AChE inhibition (Reiss et al. 2015). There are no known biological pathways for OPs or DAPs to cause the birth or neurodevelopmental outcomes examined in these studies. This does not mean that such a mechanism does not exist, but current understanding of how OPs cause toxicity and the available study data do not support causal relationships.

Specificity, experiment, and analogy: These Bradford-Hill guidelines are less informative than the others outlined above; however, they are still useful in evaluating study data. Specificity is established when a single cause produces a specific effect and provides support for a causal relationship. Most effects are likely to have multiple factors that contribute, which should all be examined. DAPs are non-specific OP metabolites, both OP and direct exposure to DAPs contribute to measured DAP levels, and many other factors can influence birth and developmental outcomes; thus, no specific relationships were observed between a specific OP and a specific outcome. Experiment implies that the outcome or effect can be altered or affected by another variable. For example, in the case of OP exposure, one might choose to look at the same outcomes in a population who followed an organic diet (no dietary OP exposure) (Reiss et al. 2015). There are no data relevant to this for OP exposures. Drawing analogies to other chemicals can sometimes strengthen the evidence of a causal relationship; in this case, analogies of OP exposure to other chemicals that cause adverse birth outcomes or developmental effects do not provide support for or against a causal relationship.

Overall, the associations reported in these human studies do not meet the Bradford-Hill criteria. Associations observed were weak and inconsistent, no clear temporal or dose-relationships were evident, associations are not biologically plausible or coherent based on available toxicological data, and they are not specific to any particular OP or birth/developmental outcome.

Conclusions and summary

Well-established and peer-reviewed science has been used to review and evaluate a large amount of epidemiologic data on low-level OP exposure in mothers and children. Based on these analyses, the evidence in these human studies does not provide support or demonstrate that low-level maternal or childhood exposure to OPs or DAPs causes adverse birth or neurodevelopmental outcomes in humans. Additional studies in this

area are required to establish any definitive relationships between OP exposure and adverse birth and/or developmental outcomes.

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