The Heartland Study is the flagship project of the Heartland Health Research Alliance, a non-profit dedicated to conducting strategic science to protect public health amidst rising pesticide use.

This multi-center, birth cohort study has been carefully designed to answer hard questions about whether moms and babies are paying the price for rising herbicide exposure. Health problems in children — including autism, ADHD, asthma, obesity and leukemia— have been linked to toxic chemicals, especially from exposures during early development. Brain-based disorders, including reductions in IQ and behavioral problems, are particularly worrisome. During pregnancy, the baby’s brain develops rapidly and continues to grow and mature through childhood, making it particularly vulnerable to chemical exposures.

The Heartland Study will measure biological and epigenetic biomarkers from mother-infant pairs to examine whether birth outcomes and developmental anomalies are triggered by herbicide exposures during fetal development.

Study Area

The Midwestern United States are ground zero for herbicide exposure. The Heartland Study will include partner clinics and hospitals across 13 states - the 12-state USGS Midwest Region plus Arkansas, an important soybean production state (Figure 1).

Millions of acres of herbicide-resistant corn and soybeans are grown in the Heartland. A remarkable 134,744,647 pounds of glyphosate, aka Roundup, the #1 herbicide in the world was sprayed on corn and soybeans in these 13 states in 2018.

Previous research by some of our scientists found glyphosate in the urine of...
over 90% of pregnant women tested, and higher herbicide levels were linked with shorter pregnancies, a concerning finding.

**Study Objectives**

- Enroll 2,000 mother-infant pairs at eight or more hospitals across the Midwest, and monitor the health and development of participating children through age 3 (and longer, pending funding).
- Collect a maternal urine sample during three trimesters of pregnancy, and quantify herbicide exposure levels.
- Quantify single herbicide and cumulative herbicide exposures and investigate associated health risks in pregnancy, fetal development, and adverse pregnancy outcomes.
- Collect cheek swabs from infants and parents for genomic analysis to assess linkages between markers of herbicide-induced epigenetic change and observed health outcomes.
- Identify risk factors for herbicide exposures among enrolled mother-infant pairs using surveys.

**Study Design**

1. **Patient Enrollment**

Expectant mothers will be enrolled at partner hospitals and birth centers throughout the 13-state Heartland Health Research Alliance (HHRA) region (see Figure 1). Each study site will coordinate recruitment and enrollment and manage entry of patient data into a Research Electronic Data Capture (REDCap) database. HHRA staff will, in turn, manage the study-wide master REDCap database, in accordance with all applicable HIPAA and IRB requirements.

2. **Study Questionnaires**

Indirect measures of herbicide exposure at two endpoints (just prior to and during pregnancy) will be analyzed and associated with urinary exposure levels. A maternal study questionnaire will be administered following delivery. Participant responses will be assessed and associated with geographical location of residence, household and work-related environments, drinking water sources, food and beverage consumption, as well as reproductive and family medical histories.

3. **Sample Collection**

Up to three urine samples will be collected from each mother during pregnancy. Urinary levels of
herbicides, including glyphosate, 2,4-D, dicamba, and atrazine, will be calculated. In addition, buccal cell swab samples will be swabbed from the inside of the cheek of moms and babies and securely stored for future analysis of potential epigenetic changes (often called epimutations). This will allow HHRA and Heartland Study scientists to correlate markers of epigenetic change to altered health and developmental outcomes, once such markers have been identified. See the figure below for an overview of the sampling schedule.

4. Medical Chart Review

Researchers will review medical charts and administer questionnaires so they can observe, document, and better understand:

- Maternal, neonatal and pediatric health outcomes;
- Any maternal risk factors or impacts from pre-existing conditions; and
- Demographic characteristics of the study population.
- Clinical outcome measurements will be collected and assessed relative to herbicide exposure levels, including:
  - Pregnancy outcomes and duration:
    - Neonatal conditions and growth percentiles; and
    - Infant and early child developmental and behavioral milestones.
    - Subpopulations will be identified based upon maternal risk factors and pre-existing conditions for separate analysis.
  - Infant and Early Child Development (Phase 2). Phase 2 is currently under development but is anticipated to include the following:
    - Infants born to a subset of moms enrolled in The Heartland Study will be monitored until at least age 3 years.
    - Children’s medical charts will be reviewed and infant demographics,
developmental progress, conditions/diagnosis, and illnesses will be recorded, as well as physiological and developmental measures such as child growth, hospitalizations, blood pressure, and BMI.

- With parental consent, infants will be scheduled for child developmental assessments at around age 3 years, whenever possible. Standardized assessment tools such as motor and cognitive screens, and ADHD and autism screens will be used and interpreted by professionals specialized in child development.
- If funding permits, children’s urine will be collected during developmental clinical visits and measured for herbicide levels.

5. Epigenome Research

The Heartland Study will assess potential herbicide-induced epimutations that may be heritable and a contributing factor in developmental problems and the onset of disease. Buccal cells are a single cell type that has been used to identify epimutations in prior studies.

We will strive to collect maternal samples at each pregnancy trimester, as well as a neonatal sample following delivery. In addition, additional buccal samples will be collected, to the extent possible, during the child’s annual developmental evaluation, and from other direct relatives (e.g., a paternal sample, grandparent samples).

Buccal cell samples will be securely stored in HHRA specimen vaults until ongoing work to identify epigenetic markers of herbicide exposure is completed and the samples can be analyzed.