Polycythemia Vera Cancer Cluster Investigation in Northeast PA

Environmental toxic substances found historically in the PV cluster area and their potential for inducing DNA damage

Investigating PV

What is PV?

PV is one of the diseases known as MPNs (myeloproliferative neoplasms). MPNs are a group of blood cancers where the bone marrow makes too many blood cells. Other illnesses included in this group of diseases are essential thrombocytosis (ET) and primary myelofibrosis (PMF).

How does the body make blood cells?

The body's bone marrow contains billions of cells, but only a very tiny group plays a key role in forming blood cells (hematopoiesis). This group is composed of hematopoietic stem cells, which provide the body with a constant supply of all types of blood cells throughout life.

What causes PV?

The cause of PV is unknown. Scientists do know that a gene mutation (called the JAK2V617F mutation or "JAK2") occurs in about 97% of PV cases. A gene mutation is a permanent change in the DNA sequence that makes up a gene inside the cells of a person's body. The causes of the JAK2 mutation are also unknown.

What is the history of the ATSDR PV investigation?

- In 2005, local physicians and community members in Carbon, Luzerne, and Schuylkill counties raised concerns about the diagnosis of four cases of PV on the same rural road in the area where the three counties come together.
- Residents also raised concerns about possible historical and current exposures to hazardous chemicals from various locations in the tri-county area.
- The Pennsylvania Department of Health asked ATSDR for help investigating the cases and pattern of PV in this area of northeast Pennsylvania.
- Using the JAK2V617F mutation test, the ATSDR investigation confirmed 33 PV cases in the tri-county area and identified a statistically significant cluster where the three counties come together. This means that the observed number of cases in this area would probably not have occurred by chance.

After the initial study, ATSDR established a collaboration with other research partners from government, academia, and private institutions to continue the PV investigation. As part of this collaboration, a toxicological assay ("tox assay") study was implemented to study the extent to which some of the chemicals found in the tri-county area groundwater in the past could have damaged specific (or 'certain') red blood cells .

What did researchers study in the tox assay study?

How did the researchers conduct these tests (assays)?

CD34 is a molecule that is present on the surface of 1–3% of bone marrow cells. A cell with this marker is called "CD34 positive" or "CD34+".

In this study, researchers used normal cord blood samples, removed CD34+ cells from the sample, and allowed them to grow in the laboratory for 48-72 hours to generate the cells that create only red blood cells.

Then researchers exposed these cells to 18 chemicals (see box) found in the tri-county area in the past and looked for damage to the cells. They tested three different concentrations of each chemical.

Researchers used two different tests, or assays, in this study:

- The **Comet assay** tested whether these chemicals caused damage to the genetic (DNA) material of these cells, and
- The **cell viability assay** tested the effect of these chemicals on the health of the cells.

Chemicals chosen for this study

- arsenic trioxide,
- benzo(a)pyrene,
- benzene,
- methylene chloride,
- 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD),
- Trichloroethylene (TCE),
- potassium chloride,
- ethylbenzene,
- benzo[k]fluoranthene,
- styrene,
- cadmium chloride,
- hydroquinone,
- 1,1,1-trichloroethane,
- sodium cyanide,
- manganese chloride,
- chromium oxide,
- lead oxide, and
- sodium arsenite.

For each of the two different assays, researchers repeated tests for each chemical at each concentration a minimum of three times.

- The researchers compared effects of varying concentrations of the different chemicals on the stem cells to any changes observed in cells that were not exposed to the chemicals (controls).

Statistics were used to determine if the amount of DNA damage and overall cell health varied with increasing levels of chemicals the cells were exposed to.

What were the results of the tests?

Most of the chemicals evaluated using both the Comet assay and the cell viability assay showed toxic effects on the cells. The DNA damage (genotoxicity) increased with increasing doses or higher concentrations of the chemical.

For the Comet assay:

- At the highest chemical concentration tested, 16 compounds showed significant damage in the exposed cells compared to the control cells not exposed to the chemical.
- Of the 18 chemicals tested, 17 showed greater DNA damage with increasing exposure concentrations.
- Of all the chemicals evaluated in this study, the DNA damage was greatest in the results for 2,3,7,8-TCDD. This chemical caused DNA damage in nearly all cells at the highest concentration tested.

For the cell viability assay:

- Overall health and vitality of the cells decreased after treatment with a chemical at higher concentrations for all except one of the 18 chemicals tested. The exception was trichloroethylene (TCE).

What do these results mean?

The two assays showed that exposure to these chemicals can cause toxicity to these special red blood cells in laboratory tests, and that damage increases as exposure levels increase.

However, this study has important limitations.

- We do not know if the concentrations of chemicals used in the current study were at all similar to actual past exposure levels for residents (including PV patients) in the tri-county area.
- While the present study indicates that some of the chemicals present in groundwater in the past do have toxic effects on cells grown and tested in the laboratory, the researchers could not determine if these effects played a role in causing (or contributing to) the observed cluster of PV patients in the tri-county area.
- Future research could use mathematical modeling to predict concentrations of chemicals in the human body that would be the same as the levels associated with toxic effects observed in the laboratory tests.

For more information

Visit ATSDR's Web page on PV: http://www.atsdr.cdc.gov/sites/polycythemia_vera/index.html

Call ATSDR's toll-free PV information line: 866-448-0242 or email jcx0@cdc.gov, which will connect you to Dr. Elizabeth Irvin-Barnwell, ATSDR Division of Toxicology and Human Health Sciences.

Contact Lora Siegmann Werner, ATSDR Region 3, by phone at 215-814-3141 or by email at lkw9@cdc.gov.