CHEMICAL MANUFACTURERS ASSOCIATION

TO: CHEMSTAR PMS

FROM: JON BUSCH Jon Busch

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RE: NTP Carcinogenicity Studies Confounded by *Helicobaiter hepaticus* Infection

For those of you who recently have had one of your chemicals tested by NTP in a cancer bioassay (or if your chemical is to be tested in the future by NTP), then the attached article may be of interest to you. Recently, as many as ten NTP cancer bioassays may have been potentially confounded by the presence of *Helicobaiter hepaticus*, a bacteria which causes liver tumors and hepatitis. NTP has not advertised which ten studies may have been impacted, but testing of one my chemicals (triethanolamine) was compromised by this bacteria.

The article makes for interesting reading.

screens suggest a chemical is unlikely to disrupt the endocrine system, EPA should label that chemical as being one of "low concern," he said. That leaves the door open to future study if additional scientific studies suggest the chemicals might interfere with hormonal activity, Koeter said.

EDSTAC's screening and testing committee presented a draft battery of screens to the full committee. After the meeting, work group leader Rochelle Tyl told *Pesticide & Toxic Chemical News* she does not know how many patented assays are in the draft battery because the group recommended types of assays rather than specific ones. But she acknowledged that there are many patented screens among the types of assays the group proposed. These include "state-of-the-art" assays developed by a California-based drug company called Ligand Pharmaceuticals, she said. Tyl directs reproductive and developmental toxicology at North Carolina's Research Triangle Institute.

Legal issues raised about patented assays

Koeter said "OECD would never adopt guidelines based on patented tests," even if the patented assays were the "best and only" ones available.

He described patented assays as "black boxes." Scientists who are not licensed to use the patented screens will know the assays' results, but not how that conclusion was reached. "You want full scientific insight," he said.

Gary Timm, senior technical advisor for EPA's Office of Prevention, Pesticides and Toxic Substances, said EPA has discussed the issue of patented assays with its Office of General Council. While EPA could not require a patented assay, the agency can include such screens on the list of those which companies can use, he told *PTCN*. Companies can select the assay of their choice from the "menu" EDSTAC eventually will recommend, Timm said. Some companies may choose to use a patented assay; others may not, he added.

Following the EDSTAC meeting, Terry Quill, a toxicologist and attorney who has been following the EDSTAC meetings on behalf of the Chemical Manufacturers Association and other clients, told *PTCN* "equivalency" is key. If the non-patented screens produce results as good as the patented ones, then the issue may not be a problem, said Quill, who works for the Washington, D.C.-based law firm Beveridge & Diamond.

If the patented assays are best, Quill said that could put small manufacturers or chemical formulators at a disadvantage, because patented screens will be more expensive.

Large companies may not want to use some patented assays, Quill added. Patent owners may refuse to license their screens, and companies would have to send their products to the owners for testing. Since pesticide and chemical manufacturing are highly competitive businesses, manfacturers may be unwilling to let a product they are developing leave their premises, he said. Quill intends to create a work group to study the legal and economic implications of patented assays.

Read this one

Carcinogenic *Helicobacter* in mice may alter human carcinogenicity tests

Scientists at the recent Toxicology Forum in Aspen, Colo., discussed the possibility that a recently discovered mouse pathogen may alter the results of rodent bioassays for human carcinogenicity and toxicity.

Jerry Rice of the International Agency for Research on Cancer chaired the panel on *Helicobacter hepaticus*, a mouse pathogen related to the human *Helicobacter pylori*. *H. pylori* causes peptic ulcers and gastrocarcinoma in humans, Rice explained, while *H. hepaticus* (first discussed in peer-reviewed literature in 1994) is associated with liver cancers and hepatitis in certain mouse strains.

In the last five years, different *Helicobacter* species have been found in different mammals, including *H. canis* in dogs and *H. pullorum* in poultry. *H. muridarum*, *H. bilis* and *H. rappini* have all been isolated in laboratory rodents. Of the rodent *Helicobacters*, however, only *H. hepaticus* has been used in culture to reproduce its associated liver diseases. *H. hepaticus* has been isolated from some wild mice as well as from lab mice sold by several major laboratories.

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According to James Fox of the Massachusetts Institute of Technology, *H. hepaticus* is a global pathogen in A/JCr strain laboratory mice, with its effects showing up somewhat more severely in male mice. Fox administered *H. hepaticus* cytotoxin to susceptible mouse strains and found that the bacterium caused chronic proliferative hepatitis which often led to liver tumors (adenomas, carcinomas, and other neoplasms) and typhilitis.

Fox also raised the possibility that *H. hepaticus* and other *Helicobacters* may be zoonotic, potentially infecting humans as well as mice, rats, or other host animals. Although he is awaiting publication of the data, Fox said his laboratory had collaborated with Chilean researchers studying high incidences of gall bladder cancer in human populations. "We have some very promising PCR data that strongly indicate they are contaminated with a *Helicobacter*," he said.

The origins of *H. hepaticus* are uncertain, although Fox suggested that previously diagnosed "idiopathic liver lesions" in mice might have been caused by *H. hepaticus* infections. But he thought it most likely that the bacterium had recently picked up virulence genes through horizontal integration. Rice agreed, noting that "I would have been surprised if it was continually missed ... and I don't believe in spontaneous generation."

Triethanolamine bioassay calls others into question

Naturally, laboratory mice already infected with *H. hepaticus* pose a problem when the mice are being used to test substances for cancer risk. But Richard Hailey of the National Toxicology Program, which runs many two-year rodent bioassays to determine toxicity and carcinogenicity, told the Toxicology Forum that the mere presence of *H. hepaticus* did not necessarily invalidate a bioassay.

"*H. hepaticus*-associated hepatitis is necessary to potentially alter the host's response to chemical administration," Hailey said. If no hepatitis is found in the mice, then the bioassay is probably accurate, since hepatitis is usually a precursor of liver cancers that result from *H. hepaticus*, he explained. NTP is currently engaged in reassessing its completed bioassays and trying to develop new methods of testing those tissue samples for *H. hepaticus*.

Dow Chemical's William Stott presented evidence that at least one NTP bioassay had been confounded by *H. hepaticus*. The chemical under consideration was triethanolamine, also known as 2,2,2-nitrilotriethanol.

Triethanolamine has been cleared for use in flume water for washing sugar beets, in pesticide formulations used before crops emerge from the soil, and in rubber and metallic articles manufacture. It can also be used in paper, paperboard, or polyurethane resins that come into contact with certain types of foods. It appears in waxes, polishes, herbicides, petroleum demulsifiers, toilet goods, cement additives, cutting oils, mineral and vegetable oil emulsions, solvents, and certain pharmaceutical aids.

A series of NTP dermal bioassays beginning in 1988 indicated that triethanolamine caused kidney tumors and other problems in male rats, liver adenomas and carcinomas in male and female mice, and no noticeable symptoms in female rats. The data from males of both species were classed as "equivocal evidence" of carcinogenicity, while the female mice provided "some evidence."

After those results came in, a Chemical Manufacturers Association-based panel on alkanolamines arranged to have the mouse tissues tested for *H. hepaticus* and found contamination in both male and female mice. The panel reported their findings to NTP, which offered to remove the male mice from the bioassay conclusions but wanted to keep the female mice results, Stott said.

"We feel that both the male and the female mice were confounded for determining cancer in the liver," Stott told *Pesticide & Toxic Chemical News*. He said that NTP now appears to be "strongly leaning toward considering the mouse [results] confounded." Stott noted that under the tiered testing system now being advocated by NTP, triethanolamine would never have reached the bioassay testing stage, since it was negative in toxicity assays.

A final technical report on triethanolamine is being printed and is scheduled to be peer-reviewed this December, according to NTP's Web site.