In the third intraperitoneal injections of Urethane (1000 mg/kg each) at 2-day intervals (i.e., on Day 1, 3 and 5), to Tg.rasH2 mice resulted in the death or the preterminal sacrifice of all males (15/15) and 13/15 females between Days 39 and 173 of the study. Microscopic examinations performed on tissues obtained from these animals revealed a high incidence of bronchiolo-alveolar adenoma and/or carcinoma and hemangiosarcoma. These findings were considered to be the cause of mortality/morbidity in the majority (22/23) of these animals.

The single intraperitoneal injection of MNU (75 mg/kg) to Tg.rasH2 mice resulted in the death or the preterminal sacrifice of 12/15 males and 11/15 females between Days 72 and 170 of the study. Microscopic examinations performed on tissues obtained from the animals revealed a high incidence of malignant lymphoma of the hemopoietic/muscular system. These findings were considered to be the cause of mortality/morbidity in the majority (28/29) of these animals.

The cause of death in each of the dose groups is summarized as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>2 Control</th>
<th>2 MNU</th>
<th>28 Urethane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of dosed mice</td>
<td>12</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>Animal weight gain</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Food consumption</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

There were no changes in body weight or food consumption that could clearly be attributed to the administration of MNU. The administration of Urethane, however, resulted in a slight and reversible decrease in body weight and food consumption during the first week of the study (Days 1 to 8) in the animals of both sexes.

CONCLUSION
In conclusion, the intraperitoneal administration of 2 known carcinogens (Urethane and MNU) to Tg.rasH2 mice followed by a 26-week holding period produced clearly higher incidences of hemangioma in tumors and tumor bearers resulting in significantly higher mortality rates among the animals treated with the carcinogens (Urethane and MNU) in comparison to Controls. Consequently, the use of the Tg.rasH2 mouse model for carcinogenicity studies is considered a suitable quicker and more cost-effective alternative to the conventional two-year mouse bioassay and is now validated at ITR Laboratories Canada Inc.

REFERENCES

ICH Guideline S1B ‘Testing for Carcinogenicity in Pharmaceuticals’.


INTRODUCTION
For several decades the two-year rodent bioassay in rats and mice has been integral part of safety testing for the carcinogenic potential of pharmaceutical agents. Unfortunately, the two-year conventional rodent bioassay is long, expensive and frequently provides ambiguous results for human risk assessment. These limitations have led to the creation of transgenic animal models, which carry genetic changes specially designed to enhance the detection of carcinogens. Regulatory agencies now agree that the carcinogenic potential of pharmaceuticals can be evaluated from data collected from one short-term carcinogenicity study using transgenic animals. In fact, the US FDA (CBER) has recommended the use of the MNU mouse for non-dermal, non-genotoxic drugs, the ITR for non-dermal, genotoxic drugs and the Tg.AC for dermal drugs. In addition, validation studies conducted in a number of laboratories around the world indicate that these transgenic models provide a quicker and more cost-effective alternative to the traditional two-year mouse bioassay, in addition to being more susceptible to carcinogens in comparison to wild-type rats.

EXPERIMENTAL DESIGN
Fifteen (15) male and 15 female Tg.rasH2 mice were assigned to each of 3 dose groups. Animals of Group 1 received purified water by oral gavage once daily for 26 consecutive weeks. Animals of Group 2 received a total of 3 intraperitoneal injections of Urethane (1500 mg/kg each) at 2-day intervals (i.e., on Day 1, 3 and 5), while animals of Group 3 received a single intraperitoneal injection of MNU (75 mg/kg). Parameters monitored during the study included mortality, clinical observations, including examinations for palpable masses, body weights and food consumption. Upon completion of the 26-week treatment/holding period (Groups 2 and 3), all surviving animals were euthanized and subjected to a necropsy examination. Subsequently, tissues collected from all animals were examined histopathologically.

A total of 2 Control, 28 Urethane-treated and 23 MNU-treated animals died or were preterminally euthanized due to poor clinical condition during the study. Bronchiolo-alveolar carcinoma of the lungs and hemangiosarcoma of the spleen were considered to be the cause of mortality/morbidity in the 2 Control females. Bronchiolo-alveolar adenoma and/or carcinoma and hemangiosarcoma were considered to be the cause of mortality/morbidity in the majority (26/28) of the Urethane-treated animals, whereas malignant lymphoma of the hemopoietic/muscular system was the cause of death/morbidity in the in the majority (17/23) of the MNU-treated animals.

The objective of the study was to evaluate the response of the Tg.rasH2 mouse test system to two known carcinogens and to demonstrate its utility as a quicker and more cost-effective alternative to the conventional two-year mouse bioassay.

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RESULTS & DISCUSSION
A total of 2 Control, 28 Urethane-treated and 23 MNU-treated animals died or were preterminally euthanized due to poor clinical condition during the study. The day and preterminal sacrifices occurred between Day 39 and 173 of the study. Mortality rates were as follows:

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number of Animals</th>
<th>Preterminal Sacrifice</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>12 (80%)</td>
<td>11</td>
</tr>
<tr>
<td>F</td>
<td>8 (53.3%)</td>
<td>7</td>
</tr>
</tbody>
</table>

Histopathological examination of the lungs and hemangiosarcoma of the spleen were considered to be the cause of mortality/morbidity in the 2 Control females. Bronchiolo-alveolar adenoma and/or carcinoma and hemangiosarcoma were considered to be the cause of mortality/morbidity in the majority (26/28) of the Urethane-treated animals, whereas malignant lymphoma of the hemopoietic/muscular system was the cause of death/morbidity in the majority (17/23) of the MNU-treated animals.

PICTURES

Bronchiolo-alveolar adenoma (HE)
Bronchiolo-alveolar adenocarcinoma (HE)
Hemangiosarcoma (HE)
Malignant lymphoma (liver; HE)