Thiazopyr increases the incidence of male rat thyroid follicular-cell tumors; however, it is not carcinogenic in mice. Thiazopyr is not genotoxic. Thiazopyr exerts its carcinogenic effect on the rat thyroid gland secondary to enhanced metabolism of thyroxin leading to hormone imbalance. The relevance of these rat tumors to human health was assessed by using the 2006 IPCS Human Relevance Framework. The postulated rodent tumor mode of action was tested against the Bradford Hill criteria and was found to satisfy the conditions of dose and temporal concordance, biological plausibility, coherence, strength, consistency, and specificity that fits with a well-established mode of action for thyroid follicular-cell tumors. Although the postulated mode of action could theoretically operate in humans, marked quantitative differences in the inherent susceptibility for neoplasia to thyroid hormone imbalance in rats allows for the conclusion that thiazopyr does not pose a carcinogenic hazard to humans.

Keywords  Human Relevance, Mode of Action, Thiazopyr, Thyroid Carcinogenesis

A number of chemical substances have been shown to induce thyroid follicular-cell tumors in rats through a mode of action that involves perturbation of thyroid hormone homeostasis via reduction of circulating thyroid hormones (Hurley et al., 1998; IARC, 1999, 2001). Homeostatic responses to low thyroid hormone concentrations result in a compensatory increase in the release of thyroid-stimulating hormone (TSH) from the pituitary gland, which in turn stimulates the thyroid gland to increase thyroid hormone synthesis and release. Persistent elevation of TSH levels leads to thyroid follicular-cell hypertrophy and hyperplasia, which if maintained (due to continuous exposure to the compound) can eventually lead to neoplasia. This neoplastic mode of action in rats is well accepted by the scientific community, and both the IARC (1999, 2000) and U.S. Environmental Protection Agency (U.S. EPA, 1998) have established specific guidance or policies for evaluating the human relevance of rodent thyroid follicular-cell tumors.

Thiazopyr, a herbicide that induces rat thyroid follicular-cell tumors by its effect on thyroid homeostasis, was the case study used to illustrate the original 2001 IPCS framework for mode
of carcinogenic action analysis (Sonich-Mullin et al., 2001). Thiazopyr’s mode of action is revisited as a case study here to illustrate the additional guidance provided in the 2006 IPCS Human Relevance Framework for evaluation of a neoplastic mode of action for humans. This updated case study highlights how accumulating experience with a particular mode of action can make subsequent analyses less difficult. Because this case study is based on an established mode of action in which the key events have been well defined, this analysis will focus on whether thiazopyr produces the biological effects expected of this pathway. This case study also emphasizes the importance of understanding the basic physiological processes underlying a toxicity pathway in animals and humans. For some compounds chemical-specific data might be critical in evaluating the key events in humans. For others, the underlying biology is sufficient to allow interpretation of the human relevance of the carcinogenic mode of action, both qualitatively and quantitatively. Thiazopyr is an example of the latter. Another mode of action case study of thyroid hormone disruption and the human relevance of rat thyroid follicular-cell tumors is available for phenobarbital (Lehman-McKeeman and Hill, in Meek et al., 2003).

The present mode of action analysis begins with a brief summary of the available information on the carcinogenicity of thiazopyr, followed by a discussion of the experimental biochemical and histopathological data considered for this thyroid disruption mode of action. It is not intended to be a comprehensive assessment of the chemical per se.

CARCINOGENICITY DATA

Human epidemiological data on the carcinogenicity of thiazopyr are not available. Thiazopyr produces effects on liver and thyroid in various laboratory species, including mice, rats, and dogs. Thiazopyr was found to induce thyroid tumors in male rats only and appears to do so by increasing the hepatic metabolism and excretion of thyroid hormones.

Chronic dietary administration of thiazopyr to mice and rats resulted primarily in thyroid follicular-cell tumors in male rats but not in female rats (Naylor and Raju, 1992; Naylor and McDonald, 1992). There were no significant increases in the incidences of any tumors in either sex in the chronic study of mice treated with up to 800 ppm thiazopyr in the diet (128.4 mg/kg/day in males and 215.9 mg/kg/day in females) (Naylor and Raju, 1992). In the rat carcinogenicity study, thiazopyr (technical, 94.8% pure) was administered to male and female Sprague-Dawley (SD) rats (60/sex/group) at dietary concentrations of 0, 1, 10, 100, 1000, or 3000 ppm, providing dose levels of 0, 0.04, 0.4, 4.4, 44.2, or 136.4 mg/kg body weight (bw)/day for males and 0, 0.06, 0.6, 5.6, 56.3, or 177.1 mg/kg bw/day for females (Naylor and McDonald, 1992). The incidences of thyroid follicular-cell adenomas and carcinomas were increased in male rats of the 1000 (44.2 mg/kg bw/day) and 3000 (136.4 mg/kg bw/day) ppm groups (Table 1). It should be noted that the increase in tumor incidence in male rats is primarily accounted for by benign tumors.

| TABLE 1 |
| Thyroid follicular-cell tumour incidence in Sprague-Dawley male rats (2-year chronic study) |
| Dose (mg/kg bw/day)* | 0 | 0.04 | 0.4 | 4.4 | 44.2 | 136.4b |
| Adenomas | 1/50 | 2/47 | 0/49 | 2/47 | 8/49 | 12/48 |
| Carcinomas | 1/50 | 1/47 | 0/49 | 0/47 | 1/49 | 4/48 |
| Combined | 2/50 | 3/47 | 0/49 | 2/47 | 9/49 | 14/48 |
| Percent | (2) | (6) | (0) | (4) | (18) | (29) |
| *p | .000c | .470 | .253 | .668 | .024* | .001** |

Note. Tumor incidences were extracted from data submitted to the U.S. EPA, Office of Pesticide Programs (Naylor and McDonald, 1992). Significance: “p < .05; **p < .01 (statistical analyses based on Fisher’s exact test).

*a mg/kg doses were estimated.

bTwo animals in the 136.4 mg/kg bw/day or 3000 ppm dose group had both benign and malignant tumours.

cFor trend with dose.

Postulated Mode of Action for the Induction of Thyroid Follicular Cell Tumours in Rats

The postulated mode of action for thiazopyr-induced thyroid follicular-cell tumors involves the perturbation of homeostasis of the pituitary–thyroid axis by an extrathyroidal mechanism. Specifically, thiazopyr induces hepatic T4-uridine diphosphate glucuronyl transferase (UGT) activity, leading to enhanced metabolism of thyroxin (T4) by conjugation and increased biliary excretion of the conjugated hormone. The result of this enhanced liver metabolism is a decrease in serum T4 (and sometimes T3) half-life. The pituitary gland responds to a decrease in circulating serum levels of T4 by enhancing the output and serum level of thyroid-stimulating hormone (TSH). Prolonged elevation of circulating TSH levels stimulates the thyroid gland to deplete its stores of thyroid hormone and continues to induce hormone production. Thus, the thyroid follicular cells enlarge (hyperplasia) and are induced to proliferate at an increased rate and to increase in number (hyperplasia). With chronic exposure, thyroid hyperplasia eventually progresses to neoplasia.

KEY EVENTS IN EXPERIMENTAL ANIMALS

The sequence of key events in thiazopyr’s mode of carcinogenic action includes:

- Induction of hepatic UGT activity.
- Increase in hepatic metabolism and biliary excretion of T4.
- Decrease in serum T4 half-life and concentration.
- Increase in circulating TSH concentration.
- Cellular thyroid hyperplaspi and follicular-cell hyperplasia.

An evaluation follows to determine whether thiazopyr works via disruption of thyroid–pituitary status by increasing hepatic
clearance of circulating thyroid hormone. Thus, based on the key events just listed, biological indicators of thiazopyr’s mode of action should include changes in liver metabolism, alterations in hormone levels, increases in thyroid growth, and lesion progression in the thyroid. These effects have been observed and measured in male rats in short term and subchronic studies, and at interim and terminal sacrifices in a chronic study (Hotz et al., 1997). The dose-response and temporal analyses of the key events and tumor response are presented next.

DOSE-RESPONSE RELATIONSHIP AND CONCORDANCE

A summary of the no-observed-adverse-effect levels (NOAELs) and lowest-observed-adverse-effect levels (LOAELs) for the key effects in thiazopyr’s mode of action are provided in Table 2. In the 56-day study by Hotz et al. (1997), male SD rats (20 per dose) were fed diets containing 0, 10, 30, 100, 300, 1000, or 3000 ppm thiazopyr (doses not measured, but estimated to be 0, 0.5, 1.5, 5, 15, 50, and 150 mg/kg bw/day) for 56 days, and evaluated for the effects on liver (weights, T4-hepatic UGT activity, T4 biliary elimination), thyroid (weights, hypertrophy/hyperplasia), and hormones (serum levels of T4, T3, rT3, and TSH). In this study, the effects on liver, thiazopyr’s primary site of action, appear to be the most sensitive indicator of pituitary–thyroid homeostasis perturbation. Statistically significant increases in hepatic T4-UGT activity in the 50 and 150 mg/kg bw/day groups (approximately 3- and 6-fold increases in activity over controls when adjusted for liver weight, respectively) were found at the end of the 56-day treatment period. Consistent with the increase in T4 UGT activity, clearance of T4 from the blood and elimination in bile (40% increase in excretion of 125I-labeled T4) were increased after 150 mg/kg bw/day of thiazopyr (only dose evaluated). Statistically significant increases in liver weight were found at 15, 50, and 150 mg/kg bw/day of thiazopyr in the 56-day study in male rats by Hotz et al. (1997). In the 2-year rat study (Naylor and McDonald, 1992), absolute liver weights were increased by 122% at 44.2 mg/kg bw/day and by 178% at 136.4 mg/kg/day relative to controls. There also were statistically significant increases in the incidence of liver hypertrophy at 44.2 and 136.4 mg/kg bw/day, (47/61 and 52/60 versus 0/60 in controls, respectively) in the 2-year rat study.

Consistent with the enhanced hepatic clearance of T4 described already, when Hotz et al. (1997) treated SD male rats with doses of thiazopyr, statistically significant (p ≤ .05) decreases in serum T4 levels (by 30%) and increases in TSH (by 60%) were found after 56 days of treatment at the highest dose tested (Table 3). T3 serum levels were nonsignificantly lower at 1.5 mg/kg bw/day and statistically significantly higher at 150 mg/kg bw/day after 56 days of treatment. In general, hepatic microsomal enzyme inducers appear to affect T3 less than T4, and thus, T4 and TSH tend to be more reliable indicators of altered pituitary-thyroid homeostasis (Hood et al., 1999; Liu et al., 1995; Hurley et al., 1998). In the case of thiazopyr, there appears to be a poor correlation between the doses causing the T4 and TSH effects and those causing an increased incidence of thyroid follicular-cell tumors. The lowest dose of thiazopyr producing a statistically significant (p < .05) increase in thyroid follicular-cell tumors in male SD rats was 44.2 mg/kg bw/day in the 2-year study, whereas the NOAEL for effects on T4 and TSH was 50 mg/kg bw/day in the 56-day study (Table 2). Generally, effects on liver enzymes/weight and pituitary–thyroid hormone concentrations would be anticipated to occur at doses at least as low as those that produce thyroid weight changes and increases in thyroid tumor incidence, given that this thyroid disruption mode of action is a threshold phenomenon. This

TABLE 2
Summary of effects on liver, hormones, and thyroid from a 56-day study (Hotz et al., 1997) and the 2-year chronic study (Naylor and McDonald, 1992) in male rats

<table>
<thead>
<tr>
<th>Effect</th>
<th>NOAEL/LOAEL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liver</strong></td>
<td></td>
</tr>
<tr>
<td>Induction of UDPG-transferase</td>
<td>15/50 mg/kg bw/day (56-day study)</td>
</tr>
<tr>
<td>Increase in T4 biliary elimination</td>
<td>&lt;150/150 mg/kg bw/day (only dose tested in 56-day study)</td>
</tr>
<tr>
<td>Increase in liver weight</td>
<td>5/15 mg/kg bw/day (56-day study)</td>
</tr>
<tr>
<td>Hepatocellular hypertrophy</td>
<td>44.2/136.4 mg/kg bw/day (2-year study)</td>
</tr>
<tr>
<td><strong>Hormones</strong></td>
<td></td>
</tr>
<tr>
<td>Decrease in serum T4</td>
<td>50/150 mg/kg bw/day (56-day study)</td>
</tr>
<tr>
<td>Increase in serum TSH</td>
<td>50/150 mg/kg bw/day (56-day study)</td>
</tr>
<tr>
<td><strong>Thyroid</strong></td>
<td></td>
</tr>
<tr>
<td>Increase in thyroid weight</td>
<td>15/50 mg/kg bw/day (56-day study)</td>
</tr>
<tr>
<td>Increase in thyroid hyperplasia</td>
<td>44.2/136.4 mg/kg bw/day (2-year study)</td>
</tr>
<tr>
<td>Increase in thyroid tumors</td>
<td>4.4/44.2 mg/kg bw/day (2-year study)</td>
</tr>
</tbody>
</table>
apparent discrepancy is probably not real, because neither of the doses quoted is accurate. In the 2-year study, the milligram per kilogram body weight doses were averaged estimates for the entire study, whereas the relevant doses for comparison with the 56-day mechanistic study are those for rats of 12–20 weeks of age. These doses would have been at least twofold higher than those that were readily available (so the real LOAEL for neoplasia would have been about 90 mg/kg bw/day). They would also have been more relevant for neoplasia because the critical period for hormonal perturbations (e.g., prolong elevation of TSH) to initiate pathological changes would be early, not late, in the 2-year study. The doses calculated for the 56-day study are also likely to be inaccurate because food intake information was not available in the publication; the doses are estimates based on assumed intakes. Having acknowledged this uncertainty, it is observed that thyroid weights were increased significantly at 50 mg/kg bw/day and liver weights were increased at 15 mg/kg bw/day, which is consistent with the liver being the initial target in thiazopyr’s mode of action.

As stated earlier, prolonged TSH stimulation leads to both hypertrophy and hyperplasia of the thyroid. In the 2-year rat study, there was a poor dose correlation between thyroid hyperplasia alone and tumor incidence. While tumor incidence was increased at 44.2 mg/kg bw/day, a statistically significant increase in the incidence of hyperplasia (8/58 versus 1/60 in controls) was found only at 136.4 mg/kg bw/day. Furthermore, in the 56-day rat study, where thyroid histology was reported as follicular-cell hypertrophy and hyperplasia combined, there was a significant increase in the incidence of this diagnosis at 150 mg/kg bw/day but not at lower doses (Hotz et al., 1997). There was, however, a good dose correlation between increases in thyroid weights in the 56-day study and tumor incidence in the 2-year study. Statistically significant increases in thyroid weights of 46% were found at 150 mg/kg bw/day and 25% at 50 mg/kg bw/day (Hotz et al., 1997). Liver weights and hepatic T4-UGT activity were increased at all observation times from the earliest time of assessment on day 7. Biliary excretion of conjugated T4 was not measured in this experiment; however, serum T4 was reduced at all observation times. Increases in circulating TSH were observed at all sampling times, although the increase was not significant at 14 days after treatment began. Increases in thyroid weight were also observed at all sampling times. Histologically, there was a time-related increase in hypertrophy/hyperplasia beginning at 14 days. In the 2-year rat study, the first thyroid adenoma was observed at week 69 at a dose of 136.4 mg/kg bw/day. Thus, there is a logical temporal response for the key events in thiazopyr-induced thyroid follicular cell tumor formation in which all key events precede tumor formation.

### Table 3

<table>
<thead>
<tr>
<th>Dose (mg/kg bw/day)</th>
<th>0</th>
<th>0.5</th>
<th>1.5</th>
<th>5</th>
<th>15</th>
<th>50</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4 (µg/dl)</td>
<td>4.1 ± 0.2</td>
<td>4.3 ± 0.3</td>
<td>3.9 ± 0.2</td>
<td>4.1 ± 0.2</td>
<td>4.0 ± 0.2</td>
<td>4.0 ± 0.2</td>
<td>2.9 ± 0.1*</td>
</tr>
<tr>
<td>T3 (µg/dl)</td>
<td>84 ± 3</td>
<td>82 ± 4</td>
<td>68 ± 2</td>
<td>84 ± 3</td>
<td>82 ± 3</td>
<td>91 ± 4</td>
<td>110 ± 6*</td>
</tr>
<tr>
<td>TSH (µg/ml)</td>
<td>2.7 ± 0.2</td>
<td>3.5 ± 0.4</td>
<td>2.7 ± 0.1</td>
<td>3.1 ± 0.4</td>
<td>2.9 ± 0.3</td>
<td>3.1 ± 0.2</td>
<td>4.3 ± 0.4a</td>
</tr>
</tbody>
</table>

*Significantly different from control with Dunnett’s test after analysis of variance (ANOVA) (p < .05).

### Strength, Consistency, and Specificity of Association of the Tumor Response with Key Events

Strength, consistency, and specificity of the association can be established from the studies described earlier. The quantifiable precursor events, fundamental to the proposed mode of action, are relatively consistent with the emergence of thyroid follicular-cell tumors. Observation of liver weight increase and induction of hepatic T4-UGT in rats receiving the thiazopyr in the diet would be consistent with perturbation of homeostasis of the pituitary–thyroid axis by an extrathyroidal mechanism. An increase in hepatic T4-UGT activity is a step occurring before the other key biochemical changes and before thyroid follicular-cell hypertrophy and hyperplasia. Thiazopyr treatment clearly results in a decrease in circulating thyroxin and an increase in TSH following enhanced liver metabolism of T4. Furthermore, in subchronic studies, the increases in thyroid weight and the development of hypertrophy/hyperplasia were shown to appear to a statistically significant degree under the same conditions of dose and time as the appearance and reversal of changes in thyroid hormone levels and thyroid hormone metabolism. Stop/recovery studies (Hotz et al., 1997) showed that cessation of thiazopyr dosing was followed by a return of hormone levels to control values, as well as a reduction in liver and thyroid weights and reversal of hyperplasia of thyroid

### Temporal Relationship

If an event (or events) is an essential element of tumorigenesis, it must precede tumor appearance. Multiple exposure time data at 7, 14, 28, 56, and 90 days are available in which male SD rats were offered diets containing 3000 ppm (150 mg/kg bw/day) (Hotz et al., 1997).
follicular cells. Early dosing withdrawal would be expected to result in a reversal of hypothyroidism and of lesion progression for this nongenotoxic mode of action. The only sign that was slow to reverse was the increase in thyroid weight after the longest dosing period.

BIOLOGICAL PLAUSIBILITY AND COHERENCE

There are considerable data from studies in laboratory rodents demonstrating the relationship between sustained perturbation of the hypothalamic–pituitary–thyroid axis, prolonged stimulation of the thyroid gland by TSH, and the progression of thyroid follicular cells to hyperplasia, hyperplasia, and eventually neoplasia (IARC, 1999, 2000; Hard, 1998; McClain, 1995; Hurley et al., 1989). Increased secretion of TSH may result via several mechanisms, including increased hepatic clearance of thyroxin, as is the case with thiazopyr.

Circulating levels of T4 are monitored by the thyrotropic cells of the pituitary gland that are responsible for the synthesis of TSH. In the pituitary gland, T4 is metabolized by 5'-deiodinase type II to T3, which then binds to specific receptors in the cell nucleus. A decrease in T3 receptor occupancy results in stimulation of TSH synthesis and secretion. Studies in vivo have shown that injection of rats with TSH leads to reductions in thyroid follicular-cell nuclear statin, a non-proliferation-specific nuclear antigen, indicating that these cells were leaving the nondividing state to resume the cell cycle (Bayer et al., 1992). This study showed that low, repeated doses of TSH (0.25 IU/rat twice daily) produced a cumulative response in nuclear statin levels over 10 days, and the response returned to normal resting levels within 5 days of cessation of TSH injections. Reduction in nuclear statin is also an early event that parallels the earliest known pinocytotic response to TSH. These data are consistent with increased TSH concentrations alone causing thyroid follicular cells of rats to enter a state of pre-proliferation. Therefore, the suggestion that thiazopyr causes thyroid follicular-cell neoplasms in rats by initially inducing hepatic T4-UGT is coherent with the known physiology of the hypothalamic–pituitary–thyroid dynamic control system, at least to the stage of hypertrophy and hyperplasia.

Lastly, the tumor response elicited by thiazopyr is typical of a rodent thyroid carcinogen in that thyroid follicular-cell tumors are found in male rats but not in female rats or mice. Rats tend to be more sensitive to thyroid carcinogenesis than mice, and male rats are frequently found to be more sensitive than female rats with respect to the proportion of chemicals that induce thyroid tumors (Hurley et al., 1998). In keeping with this, TSH levels are typically higher in male rats than in females (Hill et al., 1998). In addition, male rats are sometimes more prone to hepatic enzyme induction than females of the same strain, but this depends on the enzyme in question, the dose of the inducing compound, and the age of the animals (Agrawal and Shapiro, 1996; Oropeza-Hernandez et al., 2003; Sundseth and Waxman, 1992).

OTHER MODES OF ACTION

Mutagenesis is always one possible mode of action to consider, but no genetic toxicity has been demonstrated for thiazopyr in the following tests:

- Mutation in four strains of Salmonella typhimurium (Bakke, 1989a).
- Mutation at the hgpt locus of Chinese hamster ovary cells (Li and Myers, 1989).
- Micronucleus induction in bone marrow cells of mice treated in vivo (Flowers, 1990).
- Unscheduled DNA synthesis induction in hepatocytes of rats treated in vivo (Bakke, 1989b).

Therefore, the available evidence indicates that mutagenesis is not an alternative mode of action for thiazopyr.

Additional effects on the hypothalamic–pituitary–thyroid axis or disruption of other pathways of thyroid hormone metabolism are other possibilities of altering thyroid homeostasis. These variations would not differ in any fundamental way from the one that has been proposed for thiazopyr, in that all would lead to prolonged TSH stimulation with continuous exposure.

UNCERTAINTIES, INCONSISTENCIES, AND DATA GAPS

There appears to be a lack of dose concordance for thyroid tumors and hormone changes, but this is likely to be due to inaccuracies in the milligrams per kilogram body weight doses compared, which either were estimated (versus calculated on the basis of food consumption and body weight data) and cover an early period in the life of rats or were averages for the whole duration of the experiment, as well as experimental variability.

ASSESSMENT OF POSTULATED MODE OF ACTION

The data presented are judged, with a moderately high degree of confidence, to be adequate to explain the development of thyroid follicular-cell tumors in male rats following chronic dietary exposure to thiazopyr. Thiazopyr clearly increased liver weights (i.e., the initial target organ) at doses lower than those causing tumors and enhanced thyroid growth (i.e., increased thyroid weights) at the lowest tumorigenic dose.

Human Applicability of the Proposed MOA

The IPCS Human Relevance Framework, which was developed from the Risk Sciences Institute/International Life Sciences Institute “Human Relevance Framework” (Meek et al., 2003) and modified based on discussions by the IPCS Cancer Working Group (Boobis et al., 2006), presents a four-part approach to addressing a series of three questions and leading to a documented, logical conclusion regarding the human relevance of the mode of action underlying animal tumors.

1. Is the weight of evidence sufficient to establish a mode of action (MOA) in animals? As described in detail earlier, there is clear evidence that thiazopyr alters thyroid homeostasis by UDP
glucuronosyltransferase induction by reducing serum T4 levels and consequently elevating serum TSH.

2. Can human relevance of the mode of action be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans? The current understanding of the regulation of thyroid hormone homeostasis in humans and of the role of increased TSH levels (as a result of altered thyroid homeostasis) as a risk factor for thyroid cancer was considered in order to assess the human relevance of the key events in thiazopyr’s animal mode of carcinogenic action. Although there are substantial quantitative dynamic differences (discussed later), the fundamental mechanisms involved in the function and regulation of the hypothalamic–pituitary–thyroid axis in rats are qualitatively similar to those in humans (Bianco et al., 2002). Therefore, an agent that decreases T4 levels in rats could likewise reduce T4 in humans; this, in turn, could potentially lead to an increase in TSH levels. There are data showing that rodents and humans respond in a similar fashion to perturbations of pituitary–thyroid function. For example, it is well known that iodine deficiency, which readily leads to decreased thyroid hormone levels, stimulates thyroid cell proliferation in humans, leading to goiter. If left untreated, iodine deficiency may lead to tumor formation, albeit rarely (Thomas and Williams, 1999). Although there is no evidence of increased susceptibility to thyroid cancer, a number of pharmaceuticals (e.g., propylthiouracil, lithium, amiodarone, iopanoic acid) that disrupt thyroid homeostasis by acting directly on the thyroid gland (for example, by inhibiting hormone synthesis or release or by blocking the conversion of T4 to T3) are known to lead to hypothyroidism and increases in TSH in humans (Ron et al., 1987).

In contrast to rats, no increases in TSH levels have been found in humans following exposure to agents that induce hepatic microsomal enzymes and reduce circulating T4 levels (discussed by Lehman-McKeeman and Hill in Meek et al., 2003). For example, the pharmaceutical compounds phenytoin, rifampin, and carbamazepine induce hepatic microsomal enzymes, including UGT, and reduce circulating T4 levels, but TSH levels are unchanged (Curran and DeGroot, 1991); agents that produce thyroid tumors in rats by increasing glucuronidation and biliary excretion of T4 at high experimental doses (e.g., omeprazole, lansoprazole, and pantoprazole) produce no changes in thyroid hormones at clinical doses in humans (Masubuchi et al., 1997). Thus, there appears to be a substantial difference in the dose-response relationship for altered homeostasis of the pituitary–thyroid axis in rats compared to humans. As discussed next, this observation is due to quantitative dynamic differences between rats, and humans in the basic physiological processes underlying pituitary–thyroid function.

3. Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors between experimental animals and humans? Thiazopyr does not target the thyroid directly. Rather, its primary effect is on hepatic metabolizing enzymes, and the increase in metabolic activity indirectly increases the systemic clearance of T4, leading to the hypothyroid state and the compensatory increase in TSH found in rats. Although there are no chemical-specific data on the potential for thiazopyr to disrupt thyroid hormone homeostasis in humans, a number of other microsomal enzyme inducers have been extensively studied, such as phenobarbital (Lehman-McKeeman and Hill, in Meek et al., 2003). As discussed earlier, agents that produce hypothyroidism by altering hepatic clearance of thyroxin do not appear to result in elevated TSH levels in humans. Presumably, TSH is not increased because a critical reduction of thyroxin is not reached.

There are several important physiological and biochemical differences between rats and humans related to thyroid function. Rats have a smaller reserve capacity of thyroid hormones when compared with humans. The rat has a much shorter thyroid hormone half-life than humans. The half life of thyroxin (T4) is about 12 h in rats, compared to 5–9 days in humans (Dohler et al., 1979). The shorter half-life in rats is likely related to the absence of a high-affinity binding globulin for thyroxin that is present in humans (Hill et al., 1998). In rats, the increased clearance contributes to the need for a higher rate of production of T4 (per unit of body weight) to maintain normal levels of T4. In contrast, in humans, the binding of thyroid hormone to this globulin accounts for a slower metabolic degradation and clearance that in turn results in the thyroid gland being less active than in rats. The constitutive TSH levels are approximately 25 times higher in rats than in humans, reflecting the higher activity of the pituitary–thyroid axis in rats (Dohler et al., 1979; McClain 1992). Therefore, humans are quantitatively less sensitive than rats to agents that reduce T4 and lead to elevated TSH. There is no increased risk of thyroid tumor development if TSH is not elevated.

Another difference of rats compared to humans is the histological appearance of the thyroid. This histological difference is related to the higher rate of production of T4 to maintain a consistent serum concentration, thus making the rat thyroid more “functionally active” than primates including humans (McClain, 1995). More of the follicular epithelium in the rat is stimulated to synthesize thyroglobulin, and therefore more of the follicular cells are tall cuboidal and appear to be active in synthesis. In contrast, more of the follicular cells in humans tend to be short cuboidal or almost squamous in appearance, suggesting they are quiescent. Because rat follicular cells are already generally active, under stimulation from TSH, they will respond with hyperplasia more readily than human follicular cells. Because of the greater storage capability of the human thyroid and the greater numbers of cells in a quiescent state, human thyroid follicular cells will be roused from their quiescent state to synthesize and secrete additional thyroid hormone without the need for a hyperplastic response to reestablish homeostasis. Therefore, the primary response in the human thyroid gland would be thyroglobulin reabsorption and cellular hypertrophy rather than hyperplasia.
greater buffering capacity in the biochemistry of the human than the rat thyroid.

Even though certain agents can cause a reduction in thyroid hormone levels in humans, there is no clear evidence that these agents increase susceptibility to thyroid cancer (Ron et al., 1987). For example, epidemiologic studies with phenobarbital do not show any increase risk of thyroid cancer (Olsen et al., 1993). Studies of individuals with conditions that would lead to elevated TSH (patients with Graves’ disease or goiter) indicate the occurrence of thyroid cancer is rare in these circumstances (e.g., Mazzaferrri, 2000; Gabriele et al., 2003). A study of environmental and heritable causes of cancer among 9.6 million individuals, using the Nationwide Swedish Family Cancer Database, found that the environment did not appear to play a principal causative role in thyroid cancer (Lichtenstein and Hemminki, 2002). The only known human thyroid carcinogen is radiation, a mutagenic exposure.

As summarized in Table 4, there is sufficient evidence in the general literature on the biochemical and physiological differences in thyroid function to indicate differences in tumor susceptibility between rats and humans. In contrast to humans, rats are very susceptible to thyroid neoplasia secondary to hypothyroidism. In particular, modest changes in thyroid hormone homeostasis will promote tumor formation in rats. Thus, thyroid tumors induced by thiazopyr involving increased hepatic clearance of hormone and altered homeostasis of the pituitary–thyroid axis in rodents are considered not relevant to humans, based on quantitative dynamic differences.

### CONCLUSION: STATEMENT OF CONFIDENCE, ANALYSIS, AND IMPLICATIONS

There is sufficient experimental evidence to establish a thyroid disruption mode of action for thiazopyr-induced thyroid follicular-cell tumors in rats. Although thiazopyr may potentially result in hypothyroidism in humans, there is sufficient quantitative evidence on the basic physiological processes in the general literature to conclude that thyroid tumors induced by a process involving increased hepatic clearance of thyroid hormone and altered homeostasis of the pituitary–thyroid axis in rodents is not likely to lead to an increase in susceptibility to tumor development in humans. Although there are no human data on thiazopyr, clinical data on other hepatic microsomal enzyme inducers were critical to this human relevance analysis. The general literature provided sufficient evidence to show that unlike in the rat, decreased T4 levels typically show no evidence of compensatory increases in TSH levels in humans. There is also cellular and biochemical evidence that the rat pituitary–thyroid axis is much more sensitive than that in humans to such perturbations. This sensitivity is likely the result of the rapid turnover of T4 in rats coupled with the higher demand for TSH to maintain thyroid activity.

### IMPLICATIONS OF THE IPCS HUMAN RELEVANCE FRAMEWORK

The thiazopyr example is an illustration of an induced tumor response consistent with a mode of action that has been previously defined and established. Thus, addressing the first question

<table>
<thead>
<tr>
<th>Key event</th>
<th>Evidence in rats</th>
<th>Evidence in humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase hepatic clearance of T4</td>
<td>In short-term and chronic rat studies, the liver is found to be the most sensitive target, and evidence of increase T4 hepatic clearance is provided by studies on T4-hepatic UGT activity, T4 half-life, T4 biliary elimination, and liver weights and hypertrophy.</td>
<td>No data available for thiazopyr, but microsomal enzyme induction is plausible.</td>
</tr>
<tr>
<td>Decreased serum T4</td>
<td>Direct experimental evidence.</td>
<td>No data available for thiazopyr, but plausible given that other microsomal enzyme inducers have been shown to reduce T4 in humans.</td>
</tr>
<tr>
<td>Increased TSH levels</td>
<td>Direct experimental evidence.</td>
<td>No data available for thiazopyr, but other microsomal enzyme inducers have not been shown to increase TSH levels even when T4 is decreased.</td>
</tr>
<tr>
<td>Increased TSH increases thyroid cell proliferation and tumor formation</td>
<td>Direct experimental evidence.</td>
<td>Induction of thyroid follicular cell tumors secondary to hypothyroidism is remote in humans, given the quantitative differences in thyroid function/homeostasis. Occurrence of thyroid cancer is rare even in severely hypothyroid individuals.</td>
</tr>
</tbody>
</table>
in the framework analysis, “Is the weight of evidence sufficient to establish the MOA in animals?,” became a determination of whether the data set on the chemical conforms to the same key events defined for the pathway of interest. This example further demonstrates how data on the basic understanding of the biological processes involved in the mode of action provide an important means to compare the rodent and human key events. Thus, this generic human information was essential to evaluating the qualitative and quantitative differences between experimental animals and humans in addressing the plausibility of the cancer mode of action for humans (i.e., questions 2 and 3 in the Human Relevance Framework).

REFERENCES


