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Mode of action and human relevance of pronamide-induced rat thyroid tumors

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ABSTRACT

Pronamide, a selective, systemic, pre- and post-emergence herbicide, caused an increased incidence of thyroid follicular cell adenomas in a rat carcinogenicity study. Thyroid tumors, as well as liver and pituitary changes, were limited only to the high-dose group. The evidence for and against specific potential modes of action (MoAs) for rat thyroid follicular cell adenomas and their relevance to humans is discussed. Pronamide is not mutagenic and therefore, direct DNA reactivity is not relevant as a MoA. The hypothesized MoA for this effect is altered homeostasis of the hypothalamic–pituitary–thyroid (HPT) axis mediated by the induction of hepatic enzymes, including uridine diphosphate glucuronosyltransferase (UGT). Evaluation of data from a series of regulatory guideline and MoA studies aimed at identifying the causative and associated key events supported a UGT-mediated MoA in the development of thyroid follicular tumors. This MoA for pronamide-induced thyroid tumors in rats, which involves increased thyroid hormone metabolism/clearance, altered thyroid hormone homeostasis and HPT stimulation is not considered relevant to humans based on quantitative species differences, making rats markedly more sensitive than humans to thyroid perturbations.

1. Introduction

This review describes a weight of evidence analysis and human relevance framework for thyroid tumors seen in rats chronically exposed to pronamide in the diet. The framework of analysis used to assess the weight of evidence for MoA and the relevance of specific MoAs to humans have been described previously (Boobis et al., 2006; Cohen et al., 2004; Meek, 2008; Meek et al., 2003). The mode of action/human relevance (MoA/HR) framework increases transparency in the systematic evaluation of the available data, the weight of evidence (WOE) of hypothesized MoA(s) for critical effects, and the relevance of the hypothesized MoA to humans.

Pronamide is a selective, systemic, pre- and post-emergence herbicide. In a guideline rat chronic/carcinogenicity study, Crl:CD BR rats were given 0, 40, 200, or 1000 ppm pronamide in the diet equivalent to 0, 1.73, 8.46, or 42.59 mg/kg/day in males and 0, 2.13, 10.69, or 55.09 mg/kg/day in females for up to 24 months (Bailey, 1990a,b). There was no increase in thyroid follicular cell tumors at 6 or 12 months after initiation of exposure. However, following 24 months of exposure, there was a treatment-related increase in the incidence of thyroid follicular cell adenomas in the 1000 ppm group as shown in Table 1. There was no increased incidence of thyroid carcinomas. Furthermore, there were no thyroid follicular cell tumors at pronamide doses ⩽200 ppm, which was considered the no-observed adverse effect level (NOAEL) for the chronic toxicity study.

The hypothesized mode of action (MoA) for pronamide-induced thyroid follicular cell tumors involves hepatic enzyme induction, enhanced L-thyroxine (T4) metabolism and clearance, and activation of the hypothalamic–pituitary–thyroid (HPT) axis with elevations in thyroid stimulating hormone (TSH). Increases in TSH levels cause follicular cell hypertrophy, and with sustained TSH elevations, follicular cell hyperplasia and neoplasia occurs. Numerous compounds operate by this MoA, including phenobarbital,

Abbreviations: MoA, mode of action; HPT, hypothalamic pituitary thyroid; UGT, uridine diphosphate glucuronosyltransferase; NOAEL, no observed adverse effect level; CAR, Constitutive Androstane Receptor; PCBs, polychlorinated biphenyls; USEPA, United States Environmental Protection Agency; EDSP, Endocrine Disruptor Screening Program; PPM, part per million; TRH, thyrotropin releasing hormone; TSH, thyroid stimulating hormone; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase.

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Table 1
Thyroid tumor incidence in CD rats exposed to dietary pronamide for 24 months.[a,b]

		Male				Female			
Dose group	ppm	0	40	200	1000	0	40	200	1000
	mg/kg/day	0	1.73	8.46	42.59	0	2.13	10.69	55.09
No. thyroids examined		80	80	80	80	80	80	80	79
Follicular cell adenoma		4	2	6	**16**	1	2	1	**7**
Follicular cell carcinoma		4	2	1	4	1	1	0	0
Hyperplasia follicular, focal		1	0	1	2	0	1	0	**4**

Bolded values indicate treatment-related changes.
[a] Data extracted from unpublished pronamide rat carcinogenicity study report (Bailey, 1990b).
[b] Cumulative total values in the chronic toxicity/oncogenicity study.

carbamazepine, nicardipine, polycyclic and polyhalogenated aromatic hydrocarbons (e.g., PBB), etc. (Capen, 1992; McClain et al., 1989). The intent of this review is to describe the weight of evidence for the hypothesized thyroid MoA based on available pronamide data. The thyroid MoA is briefly described, and then data to support pronamide's effects on liver and thyroid across multiple studies will be presented. A thyroid MoA study evaluates each of the key events in the pronamide MoA. This MoA evaluation, along with additional pronamide toxicity data, is examined for strength, consistency, temporal relationship, biological plausibility, coherence, specificity and confidence in supporting the proposed MoA for thyroid tumors. The relevance of this thyroid tumor MoA to human health is discussed, along with uncertainties and data gaps. Other possible MoAs that result in thyroid tumors in rats also are examined and dismissed due to a lack of plausibility and/or supporting data. The lack of relevance of the identified MoA to humans also is described.

2. Postulated MoA for the induction of thyroid tumors by pronamide in rats

Direct DNA reactivity as a mode of action for induction of thyroid tumors was refuted because pronamide was non-mutagenic in a series of guideline studies. A battery of *in vitro* genotoxicity studies, including the bacterial reverse mutation test (Ames test), a mammalian chromosome aberration test, and a mammalian cell gene mutation test, all conducted in the absence or presence of a metabolic activation system (rat liver S9), demonstrated that pronamide does not cause gene mutations or chromosome aberrations (unpublished study reports: Brusick, 1975; Shirasu et al., 1978; Forster et al., 1984; Kumaroo and Melhorn, 1987; Wagner and Hines, 2008; results and methods are summarized in the Supplemental section). In addition, an *in vivo* mouse micronucleus assay showed that pronamide does not induce micronuclei in somatic cells or an increase in Unscheduled DNA Synthesis (UDS) in primary rat hepatocytes (unpublished study reports: Sames et al., 1984; Muller and Frank, 1987, results and methods are presented in the Supplementary section). Furthermore, the Carcinogenicity Peer Review Committee in 1992 reviewed all the *in vitro* and *in vivo* mutagenicity studies conducted with pronamide and issued a final Memorandum issued in May 1994 stating that the results of these studies indicate that pronamide does not appear to be mutagenic (U.S. EPA RED, 1994). In summary, evaluation of the genetic toxicity data for pronamide unequivocally supports a lack of DNA reactivity and hence this is not a potential MoA for the induction of thyroid tumors in rats.

Evidence to support non-genotoxic MoAs for thyroid tumorigenesis were evaluated and it was hypothesized that pronamide's induction of thyroid follicular cell adenomas involved an extra-thyroidal mechanism mediated by the induction of hepatic enzymes, including uridine diphosphate glucuronosyltransferase (UGT) as illustrated in Fig. 1. This MoA for rodent thyroid tumors is well accepted by the scientific community, including IARC (1999, 2001) and U.S. Environmental Protection Agency (U.S. EPA, 1998). The key events for this MoA are numbered in Fig. 1.

3. Overview of salient toxicity data to evaluate the proposed MoA

3.1. Pronamide thyroid function and hepatic clearance MoA study

The evidence to support the indirect UGT-mediated MoA for pronamide comes primarily from a MoA study designed to examine effects on thyroid hormone homeostasis, thyroid function, and hepatic clearance of thyroxine in pronamide-treated animals (unpublished study report, Hazelton et al., 1991; material and methods of the study are described in the Supplementary section). This study addresses each of the key events listed in Fig. 1. In this study, Crl:CD BR rats were given 0, 40, 1000, or 4000 ppm pronamide in the diet for 4 or 15 weeks (40, 20, 20, or 40 male rats/group, respectively). In addition, a separate recovery group of 20 male rats received diet containing 4000 ppm pronamide for 4 weeks followed by control diet for 11 weeks to align with the 15-week necropsy. In-life measurements included body weight, clinical observations, feed consumption, and test material intake. Endpoints assessed at or after necropsy included organ weights (liver, pituitary gland, and thyroid gland), histopathology of thyroid and pituitary, serum hormone measurements [T4, triiodothyronine (T3), reverse T3 (rT3) and (TSH), clinical chemistry (SGPT and SGOT), and UGT activity. In addition, biliary elimination of ^{125}I-L-thyroxine was measured in bile cannulated rats in the control and pronamide treated (4000 ppm) groups at 4 weeks and at 15 weeks including the recovery group.

Results from this study showed that liver weights were increased at doses of 1000 ppm (absolute (abs.) 29%, relative to body weight (rel.) 36%) and 4000 ppm (abs. 50%, rel. 91%) (Table 2). Concurrently, increased thyroid weights occurred at doses of 1000 ppm (abs. 29%, rel. 36%) and 4000 ppm (rel. 32%) following 4 weeks of treatment. After 15 weeks of treatment, liver weights were increased at doses of 1000 ppm (rel. 22%) and 4000 ppm (abs. 42%, rel. 86%). Concurrently, thyroid weights were increased at doses of 1000 ppm (rel. 21%) and 4000 ppm (rel. 33%). Neither liver nor thyroid weights were affected at 40 ppm. These data support the proposed MoA for Key Events #1 and #6.

The treatment-related increases in liver and thyroid weights were demonstrated to be reversible changes. In recovery rats, liver and thyroid weights (abs. and/or rel.) were significantly lower than those of their treated counterparts (i.e., the 4000 ppm dose group). Serum concentrations of T4 were decreased (49–87%) and concentrations of TSH were increased 40–72% at doses of 1000 and 4000 ppm at 4 and 15 weeks (Table 3). These data support key events #3 and #5. The thyroid changes were considered secondary to compound-related effects on the hepatic metabolism and biliary excretion of L-thyroxine (see below).

The biliary excretion of ^{125}I-L-thyroxine was examined using bile-duct cannulated control and high-dose rats (rats at the low