Bull. Environ. Contam. Toxicol. (1996) 56:847-852

Bulletin of Environmental Contamination and Toxicology

Acute Effects of Low Doses of Zineb and Ethylenethiourea on Thyroid Function in the Male Rat

C. Nebbia,[1] J. Fink-Gremmels[2]

[1]Department of Animal Pathology, Division of Pharmacology and Toxicology, Via Nizza 52, 10126 Torino, Italy
[2]Institute of Veterinary Pharmacology, Pharmacy and Toxicology, Yalelaan 2, De Uithof, Utrecht, The Netherlands

Received: 21 February 1995/Accepted: 12 October 1995

Zineb (zinc ethylene-bis-dithiocarbamate) belongs to ethylene-bis-dithiocarbamates (EBDC), a class of fungicides used worldwide in agriculture. They are generally characterized by low cost, good efficacy and broad spectrum of antifungal activity. In Italy EBDC are the only organic fungicides allowed to be applied by aircrafts, so that more than 11,000 tons of these compounds, either alone or combined with sulphur or copper, were employed in 1987 (ISTAT 1990). It is well established that all EBDC may give rise to ethylenethiourea (ETU) either as the result of a spontaneous breakdown, which is reported to be enhanced by heat processing of EBDC-treated foods, or following metabolic processes in both animals and plants (Lentza-Risos 1990). Impairment of haematopoiesis, fertility, thyroid function and hepatic xenobiotic metabolism are some of the most known toxic effects displayed by EBDC in animals, including food producing species (Nebbia et al. 1990; Nebbia et al. 1991; Nebbia et al. 1993). Furthermore, ETU not only retains most of the toxic effects of EBDC but has also been shown to possess mutagenic, carcinogenic, and teratogenic properties in laboratory species (Khera 1987); it is also considered a potential human carcinogen (Stein et al. 1978). Several reports indicate that residues of both EBDC and ETU may contaminate foods of vegetable (see Lentza-Risos 1990 for a review) and possibly animal origin (Gennaro Soffietti et al. 1981; Nebbia et al. 1991). Maximum residue limits in foods (MRLs) set for ETU by different countries vary from 10 to 100 ppb (Lentza-Risos 1990). In Italy, MRL values for EBDC in several foods of animal or vegetable origin have been recently lowered to 50 ppb (Direttiva 93/57 CEE 1993).

As the critical effects of either toxicant are directed towards the thyroid gland, a study was conducted to assess whether the short-time exposure of young male rats to doses of zineb or ETU even below the established MRLs is able to affect thyroid function.

Correspondence to: C. Nebbia

MATERIALS AND METHODS

Zineb (92% purity) was a gift of Farmoplant (Milano, Italy) and ETU (98% purity) was obtained from Fluka (Buchs, Switzerland). Seventy 80-90 g male Wistar SPF rats (Hagemann, Extertal, Germany) were housed in Macrolon cages (5 rats per cage) at 22 ± 1 °C and a 12-hr light/dark cycle with free access to food and tap water. They were randomly allotted to 7 groups of 10 rats each and administered a standard diet (Altromin, Lage, Germany) containing respectively 5, 50 and 500 ppb zineb or ETU for 5 days. The control group received an untreated standard diet. On the morning of day 6, after an overnight starvation, rats were weighed and euthanatized. Blood samples were withdrawn from the ocular plexus immediately prior to sacrifice and aliquots were allowed to clot to obtain sera, which were stored frozen (- 20 °C) for biochemical assays. Serum thyroxine (T_4), triiodothyronine (T_3), free thyroxine (FT_4) and thyroid-stimulating hormone (TSH) levels were determined by RIA methods (Amerlex, Amersham, Braunschweig, Germany). The concentrations of glucose and cholesterol were measured spectrophotometrically in blood and serum, respectively, immediately after collection using kits from Boehringer (Mannheim, Germany); measurement of all hormonal parameters was carried out within one week of blood collection. Data were evaluated by ANOVA followed by Student-Newman-Keul's test and the level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Both zineb and ETU tended to increase TSH levels, although the statistical significance was reached only in rats exposed to 5 ppb ETU (Fig. 1). No consistent changes in T_3 levels were observed. Zineb elicited a slight, dose-unrelated decrease in T_4 concentrations, but statistically significant changes occurred only in 50 ppb group. By contrast, FT_4 values, which represent one of the most sensitive parameters for assessing thyroid function (Kaneko 1980), were significantly depressed in 5 and 50 ppb ETU-treated rats. Results from this study agree with a previous report from Ugazio et al. (1988) indicating that ETU affects thyroid function to a greater extent than zineb. Increase in TSH and a more pronounced decrease in serum concentrations of T_4 relative to T_3 have been reported in response to much greater levels of zineb or ETU exposure in rats (O'Neil and Marshall 1984; Ugazio et al. 1988) and cattle (Gennaro Soffietti et al. 1988; Nebbia et al. 1991).

In a 90-day trial, Freudenthal et al. (1977) found that only ETU dietary concentrations exceeding 25 ppm affected circulating levels of thyroid hormones. In our study, ETU concentrations as low as 5 ppb for 5 days elicited significant changes in serum concentrations of both TSH and FT_4 but the highest doses of

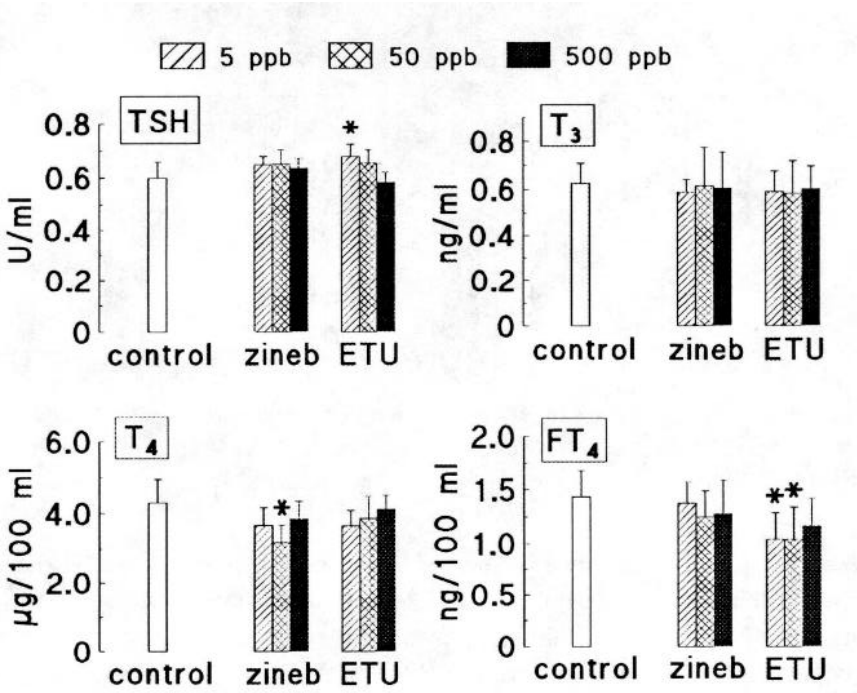

Figure 1. Changes in thyroid function in male Wistar rats orally exposed to graded amounts of zineb or ETU. Values are means ± SD, * P< 0.05 vs. control.

either compound (500 ppb) failed to alter thyroid parameters. Although many explanations are possible, the exposure to the highest doses might have led to the development of tolerance and/or the activation of detoxifying pathways (Atterwill and Brown 1987).

Zineb administration produced a marked dose-unrelated drop in blood glucose (Fig. 2). Depression of thyroid function is seldom associated with hypoglycemia as the resulting decrease in the intestinal absorption of glucose is usually counterbalanced by the reduced rate of tissue oxidative metabolism (Kaneko 1980). ETU had no effect on blood glucose. This suggests that mechanisms other than the depression of thyroid function may be at least partly responsible for the observed reduction in blood glucose. For instance, this phenomenon might be mediated by a direct action of zineb on the pancreas, where the fungicide was found to accumulate to a relatively greater extent compared to other tissues (Nebbia et al. 1987; Nebbia et al. 1991).

The measurement of serum cholesterol is used as an indirect thyroid function test (Kaneko 1980) where its concentration is inversely related with gland activity. In our study, the failure of either toxicant to increase cholesterol levels (Fig. 2) even in the presence of lowered FT_4 values might be explained by the relatively low

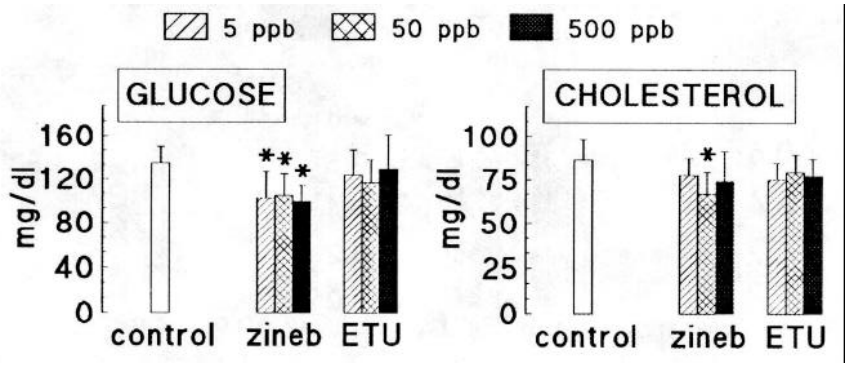

Figure 2. Effects of the oral administration of graded levels of zineb or ETU on blood glucose and serum cholesterol in male rats. Values are means ± SD, *P < 0.05 vs. control.

specificity of cholesterol measurement for determining thyroid function (Kaneko 1980). In this respect, it should be stressed that rabbits made hypothyroid by the dietary administration of 3000 ppm zineb for 90 days did not show appreciable changes in serum cholesterol levels (Nebbia et al. 1995).

In conclusion, the dietary administration of very small amounts of either zineb or ETU for as little as five days is able to elicit slight, but statistically significant, changes in thyroid function and other biochemical parameters in young male rats. When attempting to extrapolate the above results to man, it should be noted that the effects on thyroid hormones appear to be substantially reversed at the highest dosage levels (500 ppb). However, the possibility that the depression of thyroid function may be exacerbated by other factors should not be overlooked, inasmuch as conditions determining a prolonged increase in TSH output may eventually result in thyroid neoplasia (for a review see Atterwill and Brown 1987). Further research is needed to assess the possible synergism between EBDC or ETU and conditions capable of affecting thyroid function such as the dietary intake of thiocyanates or the deficiency of some micronutrients (e.g. iodine, selenium), as well as the concurrent administration of drugs showing intrathyroidal (e.g. sulphonamides, thiouracil derivatives) or extrathyroidal mechanisms (e.g. phenobarbital, omeprazole).

Acknowledgments. The Authors express their thanks to the staff of the Institute of Veterinary Pharmacology, Toxicology and Pharmacy, School of Veterinary Medicine of Hannover (Germany) for the skilful technical assistance.

REFERENCES

Atterwill CK, Brown CG (1987) Mechanistic studies on the thyroid toxicity induced by certain drugs. In: Chambers PL, Chambers CL, Dirheimer G (eds) The target organ and the toxic process. Arch Toxicol Suppl 12, 71-79

Direttiva 93/57 CEE (1993) Gazzetta Ufficiale Comunità Europea L 211, 23/81/993

Freudenthal RI, Kerchner GA, Persing RL, Baron RL (1977) Dietary subacute toxicity of ethylenethiourea in the laboratory rat. J Environ Pathol Toxicol 1: 147-161

Gennaro Soffietti M, Nebbia C, Biolatti B, Angeletti E (1981) Determinazione di residui di tireostatici in feti bovini abortiti e rilievi anatomo-istopatologici. Schweiz Arch Tierheilk 123:655-662

Gennaro Soffietti M, Nebbia C, Biolatti B, Re G, Castagnaro M, Cottino G, Guarda F (1988) Toxicology of fungicides : Effects of 270 days administration of zinc ethylene-bis-dithiocarbamate in Friesian cattle. Schweiz Arch Tierheilk 130: 657-672

ISTAT (1990) Annuario Statistico Italiano, p 459-466

Kaneko JJ (1980) Thyroid function. In: Kaneko JJ (ed) Clinical biochemistry of domestic animals. Third edition. Academic Press, New York, p 491-512

Khera KS (1987) Ethylenethiourea : a review of teratogenicity and distribution studies and an assessment of reproduction risk. CRC Crit Rev Toxicol 18: 129-139

Lentza-Risos C (1990) Ethylenthiourea (ETU) in relation to use of ethylenebisdithiocarbamate (EBDC) fungicides. Rev Environ Contam Toxicol 115:1-37

Nebbia C, Re G, Fogliato TG, Ferrero E (1987) Tossicih cronica sperimentale da etilenbisditiocarbamato di zinco nel vitello : valutazione dei residui tissutali. Ann Fat Med Vet Torino 22:51-58

Nebbia C, Re G, Gennaro Soffietti M (1990) Effects of zineb chronic administration on hepatic xenobiotic-metabolizing enzymes and liver reduced glutathione content in Friesian cattle. In: Simon F, Lees P, Semjèn G (eds) Veterinary Pharmacology, Toxicology and Therapy in food producing animals. University of Veterinary Science & Unipharma, Budapest, p 403-409

Nebbia C, Ferrero E, Valenza F, Castagnaro M, Re G, Gennaro Soffietti M (1991) Pathologic changes, tissue distribution and extent of conversion to ethylenethiourea after subacute administration of zinc ethylene-bis-dithiocarbamate to calves with immature rumen function. Am J Vet Res 52: 1717-1722

Nebbia C, Dacasto M, Gennaro Soffietti M, Rasero R, Principato GB, Di Simplicio P (1993) Inhibition of hepatic xenobiotic metabolism and of glutathione-dependent enzyme systems by zinc ethylene-bis-dithiocarbamate in the rabbit. Pharmacology & Toxicology 73:233-239

Nebbia C, Dacasto M, Valenza F, Burdino E, Ugazio G, Fink-Gremmels J (1995) Effects of the subchronic administration of zinc ethylene-bis-dithiocarbamate (zineb) to rabbits. Vet Hum Toxicol 37: 137-142

O'Neil WM, Marshall WD (1984) Goitrogenic effects of ethylenethiourea on rat thyroid. Pestic Biochem Physiol 21:92-101

Stein HP, Bahlman LJ, Leidel NA, Parker JC, Thomas AW, Millar JD (1978) Ethylenethiourea (ETU). Am Ind Hyg Assoc J 39:A34-38

Ugazio G, Boccuzzi G, Guglielmone R (1988) Failure of phenobarbitone to potentiate the adverse effects of two ethylene-bis-dithiocarbamate (EBDC) fungicides. Res Commun Chem Pathol Pharmacol 59: 191-200