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Combination effects of azole fungicides in male rats in a broad dose range

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ABSTRACT

Two 28-day feeding studies were performed in male rats to investigate combination effects of azole fungicides in a broad dose range. Following separate administration of cyproconazole, epoxiconazole, prochloraz, propiconazole, and tebuconazole at five dose levels, the first three compounds were selected to be administered in two different mixtures at three dose levels including very low doses. Here we present the data obtained by clinical observations, pathology, histopathology, clinical chemistry and haematology. The liver was the common main target organ of all compounds and their mixtures. In addition, epoxiconazole exhibited an effect on the adrenals. Furthermore, food consumption and efficiency and body weight (gain) were affected. Adverse effects of the combinations were observed at dose levels at which the individual substances caused similar effects. No evidence of adverse effects was found at dose levels below the previously established NOAELs. Our findings indicate that the concept of dose additivity appears sufficiently protective for risk assessment of the fungicides examined. Besides toxicological testing, tissue residues of the azole compounds in liver, testis and kidney were determined revealing remarkable differences following administration of the single substances and of the mixtures.

1. Introduction

In their daily diet, humans are continuously exposed to a variety of pesticide residues that might act in a cumulative way and potentially affect human health (Hass et al., 2012; Rieke et al., 2014; Cedergreen, 2014; Kortenkamp, 2014). Even though the active ingredients in plant protection products undergo rigorous and comprehensive testing before approval, it must be acknowledged that reference values are set and risk assessment is performed on the basis of experimental data that was obtained with individual substances leaving aside possible mixture effects. It is therefore not surprising that mixture toxicity is gaining increasing attention in science and regulation but also in the general public, both with regard to consumer and operator safety.

Simply due to their high number, by far not all of the presumable combinations of pesticides and their residues to which humans may be exposed can be examined on a routine basis in animals. Hence, alternative methods and concepts must be developed to facilitate cumulative risk assessment. One of the approaches is the allocation of substances to so-called "Cumulative assessment groups (CAGs)" for which additive effects are expected and that are based either on a similar mode of action or on target organs or a specific toxicological effect that they have in common (EFSA, 2013; Kennedy et al., 2015). However, experimental confirmation of this concept in vivo is still required.

One group of substances for which such a CAG has been established are triazole antifungals. Based on hepatotoxicity, a "chronic" CAG comprising 11 triazole compounds has been established (EFSA, 2009). Triazoles are widely used in plant protection and biocidal products to control fungi in many crops or to preserve wood, coatings, polymers and many other materials. Antifungal activity is related to inhibition of 14α-sterol demethylase (also known as CYP51) resulting in ergosterol depletion and subsequent disruption of cell membrane integrity (Georgopapadakou, 1998). Other compounds from the same chemical class are also applied in human and veterinary medicine to control mycoses. Both from medical use and toxicological studies, it is well known that triazole fungicides, at least when applied for a longer period and at higher doses, may exhibit severe toxic (side) effects. The

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liver was identified as the main target organ with persistent activation of the constitutive androstane receptor (CAR) and pregnane X receptor (PXR) considered a key event in pathogenesis (Nesnow et al., 2009; Peffer et al., 2007).

Additionally some triazoles may disturb steroid hormone synthesis by enzyme inhibition or can interact with steroid hormone receptors, consequently causing adverse effects in endocrine related organs (Taxvig et al., 2008; EFSA, 2009; Jacobsen et al., 2012).

Here we report the results of a 28-day feeding study in Wistar rats conducted with four triazole substances and the chemically related imidazole prochloraz individually and in combination in a broad dose range to analyse potential mixture toxicity as postulated in the CAG. Very low dietary concentrations were included to address the relevance of "low dose"-effects (Vandenberg et al., 2012; Rhomberg and Goodman, 2012). In addition to toxicological testing, tissue residues in liver, kidneys and testes were determined to elucidate possible evidence of toxicokinetic interactions and allow correlation with in vitro studies.

2. Materials and methods

2.1. Test substances

Cyproconazole (CAS no. 94361-06-5, Batch no. CHF1E00042, Purity 96.8%) and propiconazole (CAS no. 60207-90-1, Batch no. CGA64250B, Purity 96.1%) were obtained from Syngenta (Basel, Switzerland). Epoxiconazole (CAS no. 133855-98-8, Batch no. 8563,

Table 1
Dose groups, dietary concentrations and calculated mean daily intakes.

Test substances	Nominalconcen-tration [ppm]	Azole content in the diet [ppm] and deviation from nominal value [%]		Mean daily substance intake [mg/kg bw]
		Mean (±SD)	[%]	Mean (±SD)
Cyproconazole	1	0.87 (±0.24)[a]	↓ 13	0.07 (±0.00)
	10	7.05 (±0.35)	↓ 29	0.52 (±0.04)
	100	101.25 (±6.29)[a]	↑ 1	6.76 (±0.82)
	300	295.00 (±17.32)[a]	↓ 2	20.18 (±1.48)
	1000	990.00 (±14.14)	↓ 1	74.00 (±7.00)
Epoxiconazole	0.9	0.82 (±0.10)[a]	↓ 9	0.05 (±0.00)
	9	5.95 (±0.64)	↓ 34	0.41 (±0.04)
	90	83.50 (±3.00)[a]	↓ 7	5.64 (±0.67)
	270	237.50 (±35.94)[a]	↓ 12	16.78 (±1.50)
	900	860.00 (±28.28)	↓ 4	61.79 (±4.51)
Prochloraz	1	0.59 (±0.09)[a]	↓ 41	0.04 (±0.00)
	10	6.70 (±0.85)	↓ 33	0.49 (±0.03)
	100	80.25 (±14.59)[a]	↓ 20	5.45 (±0.62)
	300	217.50 (±34.03)[a]	↓ 27	15.96 (±1.54)
	1000	767.50 (±92.15)[a]	↓ 23	68.22 (±5.72)
Propiconazole	2.4	1.68 (±0.52)[a]	↓ 30	0.12 (±0.01)
	24	27.50 (±0.71)	↑ 15	1.90 (±0.11)
	240	222.50 (±15.00)[a]	↓ 7	15.18 (±1.48)
	720	665.00 (±42.03)[a]	↓ 8	46.24 (±4.56)
	2400	2150.00 (±70.71)	↓ 10	181.19 (±5.55)
Tebuconazole	1	0.82 (±0.14)[a]	↓ 18	0.06 (±0.00)
	10	8.25 (±0.21)	↓ 17	0.59 (±0.04)
	100	92.50 (±14.25)[a]	↓ 7	6.61 (±0.83)
	300	272.50 (±34.03)[a]	↓ 9	19.01 (±2.19)
	1000	990.00 (±14.14)	↓ 1	71.24 (±4.38)
Phenobarbital	500	445 (±9.3)	↓ 6	32.5 (±3.18)
Mixture I				
Cyproconazole	1	0.86 (±0.07)[a]	↓ 14	0.06 (±0.01)
	100	91.50 (±16.13)[a]	↓ 8	7.10 (±0.49)
	1000	895.00 (±123.69)[a]	↓ 10	70.22 (±10.35)
Epoxiconazole	0.9	0.78 (±0.03)[a]	↓ 14	0.06 (±0.00)
	90	77.50 (±3.11)[a]	↓ 14	6.01 (±0.41)
	900	782.50 (±35.00)[a]	↓ 13	61.39 (±9.04)
Mixture II				
Cyproconazole	1	10.50 (±5.92)[a]	↑ 950[b]	0.78 (±0.05)
	100	120.75 (±68.04)[a]	↑ 21	9.07 (±0.85)
	1000	922.50 (±205.32)[a]	↓ 7	80.63 (±13.56)
Epoxiconazole	0.9	8.75 (±3.86)[a]	↑ 827[b]	0.65 (±0.05)
	90	78.75 (±7.93)[a]	↓ 12	5.91 (±0.55)
	900	747.50 (91.06)[a]	↓ 17	65.25 (±11.00)
Prochloraz	1	0.72 (±0.20)[a]	↓ 28	0.05 (±0.00)
	100	66.50 (±8.58)[a]	↓ 34	4.99 (±0.46)
	1000	717.50 (±38.62)[a]	↓ 28	62.63 (±10.55)

[a] Based on samples taken prior to commencement and at termination of treatment.
[b] Diet preparation error.