CHLORFENAPYR (addendum)

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Explanation

Chlorfenapyr was evaluated in 2012 by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), which established an acceptable daily intake (ADI) of 0–0.03 mg/kg body weight (bw) and an acute reference dose (ARfD) of 0.03 mg/kg bw.

Studies with the metabolite tralopyril were reviewed by the 2013 Meeting, and a potency factor of 10 was established for a comparison of exposure to tralopyril with both the ADI and the ARfD of chlorfenapyr.

Following a request for maximum residue levels by the Codex Committee on Pesticide Residues, chlorfenapyr was placed on the agenda of the present Meeting, which assessed additional toxicological information on chlorfenapyr available since the last review.

A study on the in vitro metabolism of chlorfenapyr was submitted. An updated literature search was performed, and studies relevant to the assessment of chlorfenapyr were evaluated.

Evaluation for acceptable intake

1. Biochemical aspects

1.1 Biotransformation

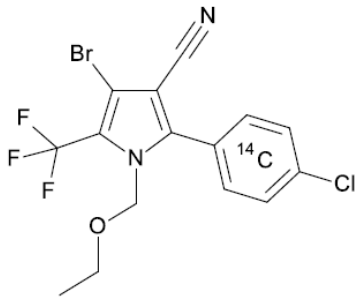
(a) In vitro metabolism of chlorfenapyr

In an in vitro study (Funk-Weyer & Kemper, 2017) that was conducted in compliance with good laboratory practice (GLP), radiolabelled chlorfenapyr (phenyl or pyrrole radiolabel; Fig. 1) was incubated with Sprague Dawley rat liver hepatocytes, the 9000 × *g* supernatant (S9) fraction from rat liver homogenate or rat liver microsomes at a concentration of 1 µmol/L (hepatocytes) or 10 µmol/L (subcellular fractions). Samples from male and female rats were mixed in an approximately 1:1 ratio prior to addition of the radiolabelled compound. Experiments were performed in triplicate. Cell viability (hepatocyte incubations) was assessed using a luminescent cell viability assay. After 0, 10, 30, 60 and 180 minutes (hepatocytes) or 0, 30, 60, 180 and 360 minutes (subcellular fractions),

ethanol was added to the incubation mixtures to stop the reaction. The samples were processed and then analysed by high-performance liquid chromatography (HPLC). Comparison of retention times, co-chromatography experiments and HPLC with mass spectrometry (HPLC-MS) experiments with reference compounds were used for the identification of parent compound and metabolites.

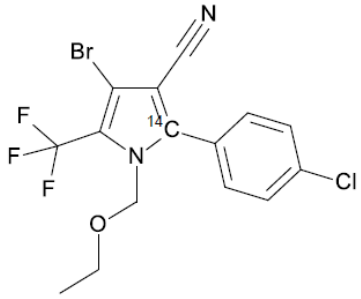
Fig. 1. Chlorfenapyr radiolabels: phenyl-U-^{14}C and pyrrole-2-^{14}C

[Phenyl-U-^{14}C]Chlorfenapyr



Specific activity: 7.64 MBq/mg
Radiochemical purity: 99.7%
Chemical purity: 100.0%

[Pyrrole-2-^{14}C]Chlorfenapyr



Specific activity: 5.28 MBq/mg
Radiochemical purity: 99.3%
Chemical purity: 98.2%

Source: Funk-Weyer & Kemper (2017)

Positive control chemicals were used to demonstrate the sensitivity of the assay to detect phase I and phase II metabolic reaction products. Appropriate negative control chemicals were used for each assay and both radiolabels.

The viability of the hepatocytes after a 180-minute incubation with unlabelled chlorfenapyr (BAS 306 I; batch no. L76-56; purity 99.6%) at 5 or 10 µmol/L was less than or equal to 53% of the viability of the cells incubated without the test item, whereas the viability was 121% at a concentration of 1 µmol/L. Therefore, a chlorfenapyr concentration of 1 µmol/L was chosen for the incubation experiments. The viability of the hepatocytes after a 180-minute incubation with radiolabelled chlorfenapyr at 1 µmol/L was 74% (phenyl label) and 105% (pyrrole label) compared with the viability without the test item. The viability studies after exposure to unlabelled or radiolabelled chlorfenapyr were performed in triplicate.

Recoveries of the applied radioactivity in the in vitro assays with chlorfenapyr were investigated for the hepatocyte, microsome and S9 experiments. For hepatocytes, the recovery in the supernatants was 71.9–95.2%. For microsomes, the recovery in the supernatants was 66.1–78.1%. The sum recovery (supernatant, extract(s) and remaining pellet) was generally around 90% (the lowest value was 85.1%). For the S9 fraction, the recovery in the supernatants was 67.3–79.2%. The sum recovery was generally around 90% (the two lowest values were 81.8%).

Chlorfenapyr was detected at all time points for hepatocytes, microsomes and S9 fraction. The concentration of chlorfenapyr generally decreased with duration of incubation.

Tralopyril was identified in almost all test systems and for both radiolabels, and its concentration as a percentage of the applied radioactivity increased with time (Table 1). There was one exception: tralopyril was not detected in the hepatocyte system after a 180-minute incubation with [pyrrole-2-^{14}C]chlorfenapyr, presumably as a result of further metabolism. The data suggest that tralopyril was formed directly from parent chlorfenapyr, as no other peaks or metabolites were detected at earlier time points. However, the existence of a transient intermediary metabolite cannot be discounted. For the phenyl label, the concentration of tralopyril increased from 2.57% to 14.89% of the applied radioactivity (hepatocytes), from 3.02% to 9.74% of the applied radioactivity

(microsomes) and from 2.75% to 8.63% of the applied radioactivity (S9 fraction); for the pyrrole label, the concentration of tralopyril increased from 1.73% to 5.30% of the applied radioactivity (hepatocytes), from 1.94% to 11.55% of the applied radioactivity (microsomes) and from 1.93% to 6.04% of the applied radioactivity (S9 fraction).

Table 1. Time course of the metabolism of chlorfenapyr (identified components)

Test system	Incubation time (min)	Chlorfenapyr[a] (% AR)	Tralopyril[a] (% AR)	Metabolite M-5[a] (% AR)
Phenyl label				
Hepatocytes	0	86.36	2.57	–
	10	90.42	4.04	–
	30	91.62	6.05	–
	60	88.42	9.75	–
	180	68.38	14.89	–
Microsomes	0	84.31	3.02	–
	30	85.85	3.36	–
	60	82.11	4.49	–
	180	80.43	6.11	–
	360	75.42	9.74	0.35
S9 fraction	0	81.73	2.75	–
	30	84.53	3.62	–
	60	83.66	4.49	–
	180	81.05	6.91	–
	360	79.84	8.63	0.48
Pyrrole label				
Hepatocytes	0	91.15	1.73	–
	10	87.81	3.08	–
	30	89.61	3.98	–
	60	93.21	5.30	–
	180	65.46	–	–
Microsomes	0	87.86	1.94	–
	30	86.08	3.92	–
	60	84.00	5.70	–
	180	79.54	8.86	–
	360	71.58	11.55	0.72

Test system	Incubation time (min)	Chlorfenapyr[a] (% AR)	Tralopyril[a] (% AR)	Metabolite M-5[a] (% AR)
S9 fraction	0	89.11	1.93	–
	30	87.54	3.17	–
	60	87.21	4.01	–
	180	85.82	5.20	–
	360	85.79	6.04	–

AR: applied radioactivity; S9: 9000 × *g* supernatant fraction from rat liver homogenate
[a] Sum % AR (mean value of triplicates) of respective supernatant and extract(s).
Source: Funk-Weyer & Kemper (2017)

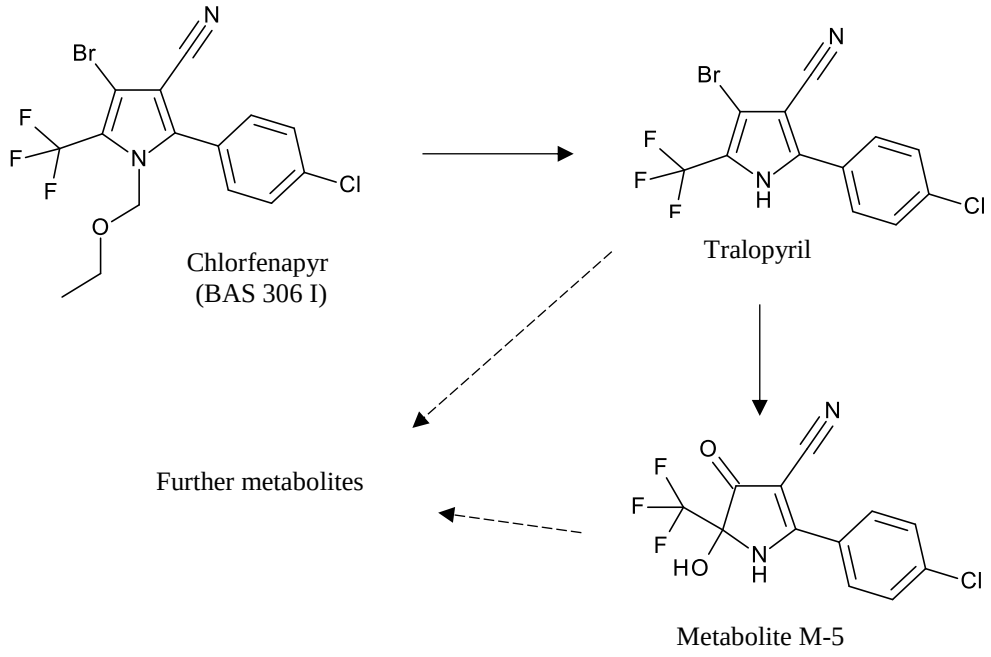
Another metabolite was detected in the chromatograms and was determined to be metabolite M-5 (reg. no. 4110561; CL325195). Its identity was confirmed via comparison of the retention times in the HPLC chromatogram and in the chromatogram of the HPLC-MS analysis and via tandem mass spectrometry (MS/MS) analysis. This metabolite was found only in very low concentrations (0.35–0.72% of the applied radioactivity) at the longest incubation time with microsomes and the S9 fraction.

Additional compounds were found at the longest incubation time (180 minutes) in hepatocytes for the pyrrole label. Most likely they are only some of the multiple metabolites that are formed from tralopyril or metabolite M-5 (Funk-Weyer & Kemper, 2017).

(b) Updated metabolic scheme for chlorfenapyr

Based on the results of the in vitro study by Funk-Weyer & Kemper (2017), the sponsor provided an updated scheme for the metabolism of chlorfenapyr (Fig. 2).

Fig. 2. Metabolic pathways for chlorfenapyr



Source: BASF (2017)

2. Toxicological studies

No toxicological studies were submitted for the current evaluation.

The sponsor provided the results of a literature search. Six online databases were searched with various terms describing chlorfenapyr and/or tralopyril. Emphasis was placed on records providing information about additional, new, unknown or potentially contradictory effects or data that might have an impact on the hazard assessment end-points or the hazard characterization and that, in addition, have a high grade of reliability (i.e. grade 1 or 2 based on the "Klimisch" scoring system).

In total, 32 studies were retrieved, of which 20 were considered relevant to the chlorfenapyr risk assessment. Reliability scores for these records ranged between 2 and 4. Only records with a score of 1, 2 or 3 (12 records) were considered potentially useful to the hazard identification or hazard characterization by the sponsor. However, upon review, it was determined that some records provided only summary information or did not provide new evidence relevant to the assessment (five records). None of the remaining seven studies (all case reports; see section 3 below) was relevant to the toxicological assessment.

2.1 Special studies

(a) Studies on metabolites

Six metabolites were identified in residue data that were not addressed by previous Meetings. These metabolites are CL152837 (M-4), CL325195 (M-5), CL322250 (M-5A), CL152835 (M-6), CL325157 (M-6A) and CL152832 (M-7A).

The sponsor provided the results of quantitative structure–activity relationship (QSAR) assessments (Derek Nexis and Organisation for Economic Co-operation and Development [OECD] QSAR Toolbox version 4.2). In both analyses, the metabolites were considered negative for genotoxicity alerts. The OECD QSAR Toolbox identified all six metabolites as Class III according to the Cramer classification method.

3. Observations in humans

Seven case reports identified in the literature search described above in section 2 described accidental or intentional ingestion or injection (one case) of chlorfenapyr formulations (Table 2). Chlorfenapyr exposure was not confirmed in any of these case reports via analytical methods. The patients died in all but one case. In general, patients exhibited extreme diaphoresis, rhabdomyolysis, neurological dysfunction and cardiac arrest prior to death. In a few cases, the neurological effects were characterized through imaging techniques, and white matter lesions were observed. These lesions are consistent with the white matter effects observed in animal toxicity studies following repeated doses of chlorfenapyr or tralopyril (Foss, 1994; Mellert, 2004). In several cases, a latency period was observed after ingestion, during which the patient did not exhibit signs or symptoms of toxicity. The latency period lasted roughly 2 weeks. Once signs or symptoms manifested, the clinical courses were similar. Sign or symptom onset, severity and latency between exposure and death appeared dose dependent where estimates of intake were possible.

Table 2. Summary of case-studies on chlorfenapyr poisonings[a]

Sex; age	Exposure	Dose estimate (mg/kg bw)	Route	Clinical observations	Outcome	Reference
Male; 55 years	250 mL, 10% chlorfenapyr	420	Ingestion	No latency before onset	Death 5 days after	Choi et al.

Sex; age	Exposure	Dose estimate (mg/kg bw)	Route	Clinical observations	Outcome	Reference
old	solution			Diaphoresis, renal failure, rhabdomyolysis, confusion, coma, fever, cardiac arrest	exposure	(2010)
Female; 28 years old	Formulation not reported	NA	Ingestion	Five-day latency before onset Progressive weakness and limb pain, swaying, drowsiness, paralysis, demyelination (MRI), coma, fever	Death 10 days after exposure	Tharaknath et al. (2013)
Male; 80 years old	200 mL, 10% chlorfenapyr solution	330	Ingestion	Three-day latency before onset Abdominal pain, diaphoresis, acute pancreatitis	Death 11 days after exposure	Huang & Lin (2015)
Female; 44 years old	10% chlorfenapyr solution (immediately expectorated)	NA	Ingestion	Fourteen-day latency before onset Bilateral leg weakness, urinary incontinence, hypoaesthesia below T11 dermatome, paraplegia, leukoencephalopathy (MRI)	Survival with unresolved paraplegia	Baek et al. (2016)
Female; 41 years old	20 mL, formulation not reported	NA	Ingestion	Fourteen-day latency before onset Nausea, weakness, drowsiness, fever, cerebral swelling and low-density white matter lesions (CT), diaphoresis, cardiac arrest	Death 14 days after exposure	Kang et al. (2014)
Male; 49 years old	200 mL, 6% chlorfenapyr solution[b]	200	Ingestion	No latency before onset Diaphoresis, dizziness, weakness, abdominal pain, tachypnoea, bilateral symmetric white matter lesions (MRI), diffuse cerebral dysfunction (EEG), fever, cardiac arrest	Death 14 days after exposure	Kwon et al. (2012)
Male; 74 years old	20 mL, formulation not reported	NA	Intraperitoneal injection	No latency before onset Diaphoresis, bowel ischaemia and sepsis, fever, cardiac arrest	Death 12 days after exposure	Lee et al. (2013)

bw: body weight; CT: computer tomography; EEG: electroencephalogram; MRI: magnetic resonance imaging; NA: not available

[a] Doses were estimated using a default body weight of 60 kg and the conversion of 1% solution = 1 g/1000 mL.

[b] Exposure was to "a combined product of chlorfenapyr and clothianidine".

Comments

Biochemical aspects

Tralopyril was shown to be the main phase I metabolite in a GLP-compliant in vitro study on the metabolism of chlorfenapyr in rats (Funk-Weyer & Kemper, 2017). In the context of the data previously reviewed by JMPR, this study provides assurance that rats exposed to chlorfenapyr are also exposed systemically to tralopyril. It was not possible to estimate quantitative exposure to this metabolite in vivo from this study.

Toxicological data

No new information on the toxicity of chlorfenapyr or tralopyril was submitted to the 2018 Meeting, nor was any new information identified in the open literature.

Toxicological data on metabolites and/or degradates

Six metabolites were identified in residue studies that were not addressed by previous Meetings and were of potential relevance for the residue definition for risk assessment. These metabolites are CL152837 (M-4), CL325195 (M-5), CL322250 (M-5A), CL152835 (M-6), CL325157 (M-6A) and CL152832 (M-7A). No toxicological data were submitted for these metabolites, but they did not show any alerts for genotoxicity. For chronic toxicity, the threshold of toxicological concern (TTC) approach can be applied (Cramer class III).

Human data

Several case reports were identified in the scientific literature that described human poisonings after self-reported ingestion or, in one case, intra-abdominal injection of chlorfenapyr solutions. Neurological signs and symptoms (diaphoresis, dizziness, weakness, fever, paralysis) were common and progressed in severity until death by cardiac arrest in all but one case (Choi et al., 2010; Kwon et al., 2012; Lee et al., 2013; Tharaknath et al., 2013; Kang et al., 2014; Huang & Lin, 2015; Baek et al., 2016). In some cases, imaging techniques were used to characterize white matter lesions in the patients, which appeared consistent with the effects noted after chlorfenapyr or tralopyril exposure in rats (Foss, 1994; Mellert, 2004). In cases where an estimate of dose was feasible, lethal doses appeared to range from 200 to 420 mg/kg bw (compared with the rat median lethal dose [LD_{50}] of 441 mg/kg bw; Lowe, 1993). The Meeting noted that the ARfD provided a margin of four orders of magnitude relative to these estimated lethal doses.

The human clinical data suggest that, compared with rats, humans are of similar or greater sensitivity to the acute effects of chlorfenapyr, and the toxicological consequences to the nervous system are very similar. There is no information available on the comparative metabolism of chlorfenapyr in rats and humans.

Toxicological evaluation

The Meeting concluded that no revision of the ADI or ARfD was necessary.

The Meeting also concluded that six metabolites identified in residue studies – CL152837 (M-4), CL325195 (M-5), CL322250 (M-5A), CL152835 (M-6), CL325157 (M-6A) and CL152832 (M-7A) – were toxicologically not relevant at currently estimated dietary exposures.

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