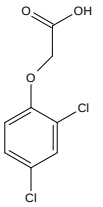
5.9 2,4-D (020)

RESIDUE AND ANALYTICAL ASPECTS

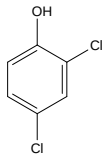
The herbicide 2,4-D, 2,4-dichlorophenoxyacetic acid, is registered in a variety of salt, amine, and ester formulations for control of broadleaf weeds in a variety of food and feed uses. As a synthetic auxin herbicide, 2,4-D causes disruption of plant hormone responses. It is currently registered in many countries.

It was first evaluated by JMPR in 1970. It was subsequently evaluated in 1986, 1987, 1996, 1997, 1998 and 2001. The 1998 JMPR evaluated it under the Periodic Reevaluation Programme. Its specification was established by the Joint FAO/WHO Meeting on Specifications in 1994. The present ADI is 0–0.01 mg/kg bw for sum of 2,4-D and its salts and esters expressed as 2,4-D, and ARfD is unnecessary. The present residue definition established by the 1998 JMPR is 2,4-D for enforcement of MRLs and for dietary intake estimation for plant and animal commodities. The residue is not fat-soluble.

2,4-D was scheduled for JMPR evaluation in the year 2017 for consideration of residues arising from a GM cotton crop. There are a number of Codex MRLs but no MRL has been set for cotton seed.



2,4-D



2,4-DCP

Plant metabolism

The 1998 JMPR evaluated plant metabolism studies on apple, lemon, potato and wheat. The predominant residue was 2,4-D in these plants, except in the apple study in which radioactivity was too low to identify.

The current Meeting received information on GM cotton, in which expression of the α-ketoglutarate-dependent aryloxyalkonoate dioxygenase-12 (AAD-12) protein confers tolerance to 2,4-D and associated increased metabolism of 2,4-D (hereafter referred to as AAD-12 cotton).

A plot of AAD-12 cotton was treated with [^{14}C-]-2,4-D choline (2,4-dichlorophenoxy acetic acid choline salt labelled at phenyl ring) at the maximum seasonal rate in the USA of 3.3 kg ai/ha (three applications each at 1.1 kg ai/ha at pre-emergence, BBCH 61, and BBCH 65 with an interval of 12 days). The 2,4-D choline was formulated as a soluble concentrate. The cotton was grown outdoors to maturity. Cotton bolls were harvested when the lower bolls were mature, unlike in commercial production.

Total radioactive residue (TRR) 56 days after the last application was 1.2 mg eq/kg in cotton seed and 40 mg eq/kg in cotton trash.

A series of extractions with hexane, methanol/water (9:1) and methanol/2M NaOH extracted 32% TRR in seed and 83% TRR in cotton trash (no hexane extraction). Refluxing in 2M NaOH further extracted 32% TRR in seed and 12% TRR in trash; and refluxing in 2M HCl extracted 3% TRR from seed. After these treatments, the unextracted radioactivity was 31% TRR in the seed and 3.4% TRR in trash. The unextracted radioactivity was attributed to pectin, lignin, acid-detergent fiber and cellulose. Overall, 98% of the applied radioactivity was recovered from the seed and trash.

2,4-D was metabolized into numerous components and a number of radioacitive components were identified. Major residues identified were parent 2,4-D and conjugates of 2,4-DCP. Other metabolites were also identified but at lower rates of <5% TRR.

Cotton seed: In the methanol/water extract, 2,4-D accounted for 4.8% TRR (0.057 mg eq/kg) and free and conjugated 2,4-DCP accounted for 8.3% TRR (0.099 mg eq/kg), mostly in the form of sulphate-glucose conjugate (4.7% TRR). In the methanol/NaOH extract, 2,4-D accounted for less than 0.1% TRR (0.001 mg eq/kg) and free and conjugated 2,4-DCP accounted for around 2.7% TRR (0.032 mg eq/kg). In these extracts, hydrolysis released free 2,4-DCP effectively and hydrolysed 2,4-D to a certain extent to produce 2,4-DCP. 2,4-D and free and conjugated 2,4-DCP were below 2% TRR in the 2M NaOH extract.

Cotton trash: In the methanol/water extract, 2,4-D accounted for 31% TRR (12 mg eq/kg) and free and conjugated 2,4-DCP accounted for 33% TRR (13 mg eq/kg), mostly in the form of sulphate-glucose conjugate (23% TRR). In the methanol/NaOH extract, 2,4-D accounted for 2.5% TRR (0.99 mg eq/kg) and free and conjugated 2,4-DCP accounted for around 2.5% TRR (0.98 mg eq/kg). In these extracts, hydrolysis released free 2,4-DCP effectively and hydrolysed 2,4-D to a certain extent to produce 2,4-DCP. 2,4-D and free and conjugated 2,4-DCP were below 2% TRR in the 2M NaOH extract.

The metabolism of 2,4-D in conventional crops (lemon, potato and wheat) evaluated by the 1998 JMPR and that in 2,4-D tolerant transgenic cotton (AAD-12 cotton) were qualitatively similar. Quantitatively, while in conventional crops 2,4-D was the predominant residue with 2,4-DCP as one of the minor components, in AAD-12 cotton 2,4-DCP and its conjugates were detected at similar or even at higher ratios of the TRR. However, 2,4-DCP may occur in or on the plants not only from the use of 2,4-D, but also from its presence in water and the environment and, therefore, it was not considered appropriate to include this compound in the residue definition.

The main route of metabolism of 2,4-D in AAD-12 cotton is the rapid loss of the acetic acid side chain to produce 2,4-DCP. 2,4-DCP is also rapidly conjugated with glucose, which is subsequently further conjugated. Metabolism proceeds through natural incorporation of the radiolabelLed carbon into natural plant constituents, such as lignin and cellulose.

Due to its chemical structure, 2,4-D is not expected to concentrate in cotton seed oil, crude or refined. It was not possible to calculate processing factors as 2,4-D was <LOQ in cotton seed and not detected in crude or refined oil.

Methods of analysis

Analytical methods for 2,4-D and 2,4-DCP in undelinted seed and gin by-products of AAD-12 cotton was submitted to the current Meeting. It was concurrently validated within the supervised residue trial studies.

The analytical procedure involves extraction of 2,4-D and 2,4-DCP with methanol/1M NaOH (90:10, v/v). After addition of stable-isotope internal standards, and concentration to near dryness, the sample solution was incubated at 90 ± 5 °C for a minimum of 60 minutes in the presence of 2M HCl. The samples were analysed using an online reverse-phase polymeric solid-phase extraction (SPE) cartridge with liquid chromatography with negative-ion electrospray tandem mass spectrometry (LC-MS/MS).

The validated LOQ of the method was 0.01 mg/kg for 2,4-D and 2,4-DCP in undelinted cotton seed and cotton gin by-products.

Stability of residues in stored analytical samples

Freezer storage stability of 2,4-D and 2,4-DCP in samples of AAD-12 cotton and its processed fractions were studied at the fortification level of 0.10 mg/kg at -20 °C for a duration of 3 to 8 months.

The results of the study indicate that residues of 2,4-D and 2,4-DCP are stable in cotton gin by-products for at least 6 months and stable in cotton hulls, untoasted meal, toasted meal, crude oil,

and refined oil for at least 3 months when stored at -20 °C. The results of the study indicate that residues of 2,4-D and 2,4-DCP experienced some observable degradation in cotton undelinted seed during storage of one month and three months, respectively.

Results of supervised residue trials on crops

The Meeting received supervised trial data for 2,4-D on AAD-12 cotton.

Due to the questionable storage stability of both 2,4-D and 2,4-DCP in cotton seed, it was not possible for the Meeting to evaluate the trial data.