DEGRADATION OF BROMOPHOS IN STORED WHEAT

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ABSTRACT: In this paper experiments on the degradation of bromophos (0,0-dimethyl-0- 2,5-dichloro-4-bromopheny! thionophosphate) and its distribution in grain and ground products are described.

At various intervals during the storage period samples were taken and analysed by gas chromatography for bromophos. The wheat was also examined for the bromozone, the corresponding oxygen isolog, and for the hydrolytic degradation product 2,5-dichlor-4-bromophenole.

The initial amounts directly after treatment range from 5.5 to 7 ppm at an application rate of 8 ppm spraywash or dust. At an application rate of 12 ppm the initial amounts are between 9.5 - 10 ppm. After 1 year amounts of 3.5 - 4 ppm (application rate of 8 ppm) and 5 - 6 ppm (application rate of 12 ppm) are found.

The type of formulation and the degree of moisture content of the stored grain have no significant influence on the degradation of bromophos. However, by increasing storage temperature, the rate of decomposition is significantly increased.

Of the above mentioned metabolites only the corresponding phenole is detectable. After 1 year up to 1 ppm can be found.

On examination of the ground products, it was established that fractions of the active ingredient pass through outer layers of the grain and move into tissues lying underneath. It appears that an increase takes place in the aleurone layer, where the fats and lipids hold the lipophilic bromophos.

Thus only relatively small amounts of bromophos are to be found in the flour, which after 1 year lie between 0.8 and 1.2 ppm. The residues are greatly reduced during the making of bread resulting in values up to approximately 0.1 ppm bromophos. On the other hand in bread made from coase meal the residues after 6 - 12 months are about 2 ppm.

The bread made from flour and coarse meal were examined for odour and flavour. Any disadvantageous influence which could be traced back to the bromophos treatment was not observed.

INTRODUCTION: Bromophos (0,0-dimethyl-0-2,5-dichloro-4-bromophenyl

thionophosphate) is an organophosphate with very low short-term toxicity [1]. Because of the broad insecticidal efficacy and rapid degradation [2] the formulations are widely used in crop protection. Another paper [3], presented at this symposium, describes the application of bromophos as a grain protectant.

In the past, several authors have engaged in studies on degradation and metabolism of bromophos in grain [4]. In extensive laboratory trials Rowlands [5], [6] treated wheat (up to 10 g samples) with 10 ppm of bromophos. A high rate of degradation could be shown within the first few days; in the following period (break-down of approx. 75% in 10 weeks), however, a "stepping effect" was observed. The author supposed that desmethyl bromophos (O-methyl-O-2,5-dichloro-4-bromophenyl thionophosphate), the primarily formed main metabolite, temporarily inhibited alkyl phosphatase, which is probably involved in the hydrolysis of bromophos. Since this metabolite disappeared during the following 2 - 3 weeks the enzymatic degradation of the parent compound could be continued, producing further amounts of desmethyl bromophos. This rhythm repeated itself in the course of the further degradation. Horler and Clarke [7] demonstrated that bromophos is rapidly degraded by microorganisms (laboratory cultures of *Alternaria* and *Aspergillus* spp.). During their investigations they also observed the "stepping effect".

As further relevant metabolites, Rowlands [5], [6], [8] found 2,5-dichloro-4-bromophenol and traces of bromoxone (O,O-dimethyl-O-2,5-dichloro-4-bromophosphate) at the beginning of the trials. In our metabolism studies, which were limited to wheat, only the toxicologically relevant bromoxone and also the dichlorobromophenol have been considered; the latter is of interest because of the significant amounts produced.

Green et al. [9] studied the biological activity and the degradation in wheat and barley stored under varying conditions. It could be shown that bromophos residues assayed up to 36 weeks were more rapidly reduced in bagged wheat and barley stored at 25° C than in cooled wheat stored during winter in bulk on a farm.

Field scale trials were also conducted on maize treated with 10 ppm bromophos. After 40 - 50 weeks residues of about 1 ppm or less were found [10].

This paper describes large-scale laboratory trials to determine the degradation of bromophos depending on different experimental procedures. Type of formulation, moisture content of the wheat and storage temperature were varied. Storage conditions, the subsequent milling of the grain and the baking process were simulated to usual practice.

EXPERIMENTAL PROCEDURE: Treatment and storage - A wheat containing 60% German wheat and 40% Manitoba wheat, typically used for baking, was chosen. The samples had been harvested about 5 months before starting the experiments. The administered "bromophos pure" was a representative specimen of the bromophos which is produced on a technical scale for application in the hygiene and storage sector. The active ingredient was formulated as dust (1.2%) or as emulsion

concentrate (40%). Both formulations were added to the grain in a mixer at concentrations of 8 and 12 ppm bromophos. Each series of experiments comprised 100 kg and had a moisture content of 13.5%. After a part of the wheat treated with 12 ppm active substance (as emulsion) was withdrawn in order to use it for supplementary experiments, the samples were poured into large open containers which were then stored in a dry room under indirect daylight. The average storage temperature amounted to 15°C (minimum in winter: 9°C ; maximum in summer: 22°C).

The wheat set aside for the supplementary trials was divided into two parts. One sample was moistened and equilibrated to 15% moisture content; the other was held at 13.5%. These two samples were each poured into open containers which were stored in a room under natural lighting conditions at an average temperature of 26°C (variation between 22° - 32°C).

Directly after treatment and 3, 6 and 12 months later samples were prepared by mixing grain withdrawn from different depths of the containers. These samples were set aside for milling and analytical assay.

Milling and baking process - The wheat (approx. 5 kg) intended for baking was cleaned, conditioned (equilibration to 16% m.c.) and after one week milled in a laboratory mill (model: Quadrumat-Junior), giving a flour similar to type 550 (0.55 g ash/1 kg flour). This milling process simulated the conditions usually present in mill practice. Samples of flour, coarse bran and semolina bran were set aside for residue assay.

Crushed grain and flour (sampling date: 3,6 and 12 months) were each baked, adding yeast, common salt and water (approx. 500 g in 1500 g dough). 3 and 24 hours after baking the loaves were cut into pieces and examined for odour and flavour by 5 - 6 test persons, using bread made with the milled control wheat as comparison.

Analytical methods - The hormogenized samples were extracted with acetone or methyl alcohol. The first clean-up step was a partition to hexane. For the determination of bromophos or bromoxone one part of the extract was chromatographed on florisil or polyamide respectively. The quantitative analyses were carried out by gas chromatography [11] [12]. The other part of the hexane extract was used for the analysis of dichlorobromophenol. Adding acetic anhydride dichlorobromophenyl acetate was formed. This was chromatographed on a florisil column and determined by gas chromatography using an electron capture detector.

The average recoveries amounted to 95% for bromophos and bromoxone and 85% for dichlorobromophenol. The residue data, summarized in the tables, are mean values of two analyses and are corrected for 100% recoveries. The lower determination limit was between 0.005 - 0.01 ppm.

RESULTS: Degradation rates in wheat grains - The residue data of bromophos in wheat grains stored at a lower average temperature of 15°C (range of 9° - 22°C), are summarized in table 1. The results

TABLE I. Degradation of bromophos (ppm) in wheat grains[a]; comparison of dust and emulsion.

months after applic.[b]	applic. of 8 ppm a.i.		applic. of 12 ppm a.i.	
	dust	emulsion	dust	emulsion
0	6.8	5.4	10.4	9.4
3	4.3	5.6	7.2	9.2
6	4.3	3.5	6.7	6.2
12	4.1	3.4	6.1	4.7
degrad. after 1 year	40%	37%	41%	50%

[a] storage temperature at 15°C
[b] application in February.

show that the main breakdown, amounting to about 35%, occurs during the first six months. This period also included the months with higher temperatures which probably increase the activity of degradation. In the second period of these trials bromophos breaks down less rapidly. After 12 months' storage the degradation rate is about 40 - 50% (emulsion: 12 ppm) and the initial deposit of 6.8 or 5.4 ppm (dust or emulsion: intended 8 ppm a.i.) is reduced to 4.1 or 3.4 ppm. Corresponding to the higher application rate (intended 12 ppm a.i.) bromophos residues fall from 10.4 or 9.4 ppm to 6.1 or 4.7 ppm. The type of formulation has no significant influence on the degradation rate after one year but there is some evidence that the initial breakdown is different.

As was seen in supplementary experiments (emulsion: 12 ppm a.i.) bromophos residues (table II) decrease much faster when storage temperatures are increased; at 26° C about 60% of the parent compound are degraded, the corresponding values at 15° C were 40 - 50%. It is obvious that the higher temperature effects an increase in the enzymatic activity; especially the reduction of bromophos content within the first three months is much more pronounced. The different moisture content of 13.5% or 15% does not influence the rapidity of bromophos reduction. In both series the residue data are very similar.

Distribution of bromophos in milled products - Rowlands [5] [6] has already described the rapid penetration of bromophos through the pericarp. We have found similar results. In order to present a clear picture, table III includes only results of 3 month and 12 month storage periods (application of dust and emulsion).

The coarse bran mainly consists of outer layers of the grain (inner pericarp and seed coat); besides parts of outer layers and some flour, the semolina bran contains main parts of both the aleurone layer surrounding the starchy endosperm, and the germ.

TABLE II. Degradation of bromophos (ppm) in wheat grains[a] with varying moisture content and under varying storage temperatures.

months after applic.[b]	m.c. 13.5% temp. 15°	m.c. 13.5% temp. 26°	m.c. 15% temp. 26°
0	9.4	9.4	9.4
3	9.2	5.7	5.4
6	6.2	5.0	4.7
12	4.7	3.6	3.7

[a] application of 12 ppm a.i. as emulsion
[b] application in February

TABLE III. Distribution of bromophos (ppm) in milling products (application rate 12 ppm a.i.).

months after applic.	whole[a] grain	coarse[b] bran	semolina[c] bran	flour
		application as dust:		
3	7.2	22.0	21.2	2.34
12	6.1	14.3	9.4	1.17
		application as emulsion:		
3	9.2	23.5	22.7	2.66
12	4.7	14.1	10.1	1.10

[a] crushed just before analysis in a grinder
[b] consists mainly of inner pericarp and seed coat
[c] consists besides inner pericarp, seed coat and flour, of main parts of aleurone layer and germ.

After 3 months considerable amounts of bromophos can be found in coarse bran (22.0 or 23.5 ppm) and semolina bran (21.2 or 22.7 ppm). Over 9 months the values for coarse bran fall to 14.3 or 14.1 ppm, amounting to a reduction of 35 - 40%. The corresponding degradation rate for semolina bran is significantly higher and reaches more than 55%; the bromophos content decreases to 9.4 or 10.1 ppm. On the given sampling dates there are no differences in the penetration rate for the dust or emulsion application.

The bromophos content found in coarse bran includes the deposit on the grain surface and the portions which penetrated into the pericarp and seed coat. From this tissue parts of the parent compound can enter the aleurone layer and the germ. In these cells lipophilic plant ingredients are increased which probably absorb the likewise lipophilic bromophos molecule. It seems that the aleurone layer has the function of a barrier causing only

slight distribution of the parent compound to the starchy endosperm. Compared with the data assayed for the bran fractions the content in flour is approx. 1/10.

The results in table III demonstrate a pronounced breakdown of bromophos in the different areas of the grain, apparently catalysed by hydrolysing enzymes. Rowlands [8] found esterase and phosphatase activity in the aleurone layer as well as in the scutellum of the germ and from these studies the higher degradation rate in the semolina bran compared with that in the coarse bran could be explained.

Analytical assay of degradation products - The analyses were carried out on grains sampled 6 or 12 months after application of dust or emulsion. In no case is bromoxone detectable. As was seen by Rowlands [6] only traces of bromoxone were found in grains within the first 3 - 4 weeks of storage. He concluded that there is only slight oxidation of bromophos. This statement coincides with results of metabolism studies on other plants carried out in our laboratories [2].

2,5-Dichloro-4-bromophenol is produced as the predominant metabolite (table IV). The assayed data range from 0.23 - 0.40 ppm (application of 8 ppm a.i.) or from 0.44 - 0.80 ppm. The fact that

TABLE IV. Residues of 2,5-dichloro-4-bromophenol (ppm) in wheat grains.[a,b]

months after applic.	application of 8 ppm a.i.		application of 12 ppm a.i.	
	dust	emulsion	dust	emulsion
6	0.35	0.23	0.44	0.49
12	0.40	0.39	0.70	0.80

[a] moisture content: 13.5%
[b] storage temperature: 15°C

higher residues of the phenol compound occur after the longer storage period has already been observed in the past [6]. There is some evidence that the degradation of dichlorobromophenol is prolonged and the values increase because of further hydrolysing processes within the experimental period.

Experiments on bread - The bread contamination rate is of fundamental interest since bread is the main food processed from grain consumed by man. The results in tables V and VI prove that large amounts of bromophos disappear during baking. It seems clear that after adding water the high temperature effects decomposition by hydrolysis and probably further residues are diminished by evaporation. After the baking of crushed wheat only about 25 - 35% of the bromophos originally contained in the grain are recovered (table V). In bread made with flour the corresponding data show a disappearance of even 85 - 90% (table VI). It is noteworthy that the rate of reduction is more pronounced in bread prepared

TABLE V. Comparison of bromophos residues (ppm) in crushed grain and bread.

months after applic.	application of dust[a]		application of emulsion[a]	
	crushed grain	bread	crushed grain	bread
3	7.2	1.59	9.2	2.48
6	6.7	1.73	6.2	2.05
12	6.1	2.23	4.7	1.40

[a] application of 12 ppm a.i.

from flour; the larger particles of the crushed wheat include parts of the parent compound apparently making the breakdown more difficult. After 3 - 12 months bromophos residues in bread made with crushed wheat range from 2.5 - 1.4 ppm and in bread made with flour only traces of 0.3 - 0.1 ppm are detectable.

After having reported all detailed data, a graphic presentation of one typical series (application of 12 ppm a.i. as emulsion) may facilitate the concluding statement that bromophos shows favourable degradation behaviour (figure 1). Besides the breakdown during grain storage the most predominant disappearance occurs during the processing of flour from wheat and during baking.

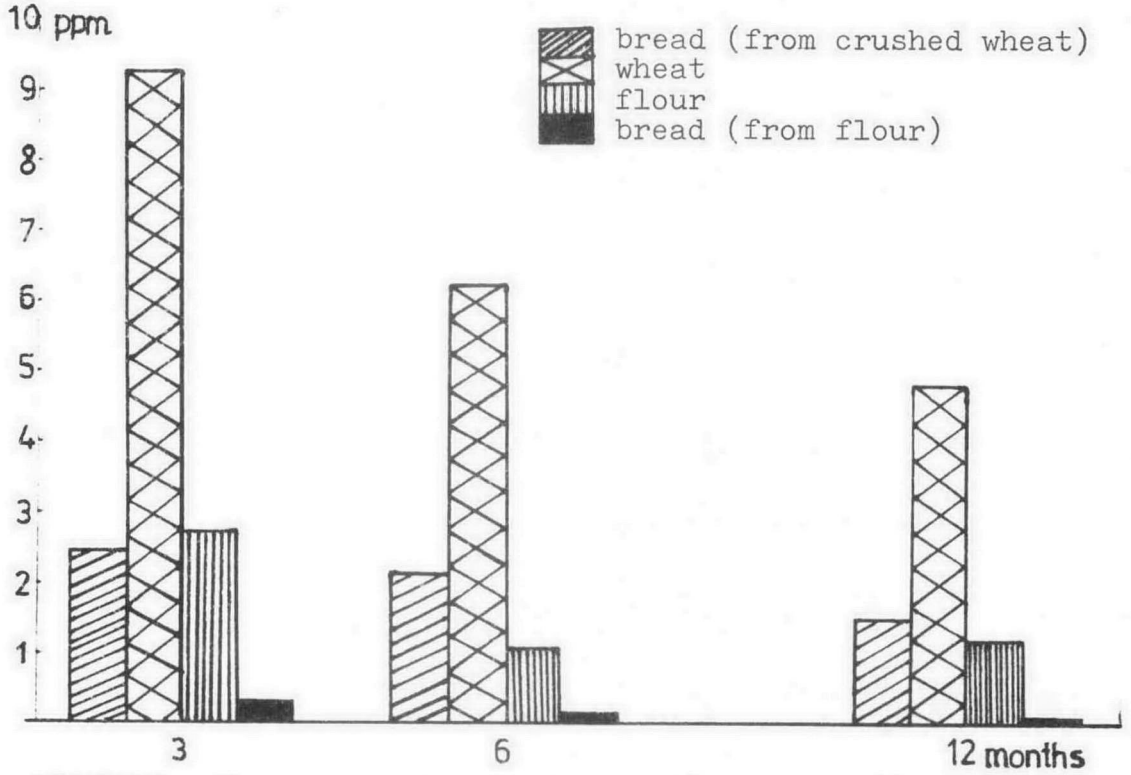

FIGURE 1. Comparison of bromophos residues in wheat, flour and bread (application rate: 12 ppm a.i. as emulsion; storage temperature of the wheat: 15° C).

Since the type of bread prepared from flour has greatest distribution world-wide, it is the most important food processed from wheat for human beings and in this connection it is of interest that the bromophos residues are only about 2 - 3% compared with those found in the grain used.

Furthermore the loaves were examined as to whether their odour and flavour could be classified as "typical for bread" or as "strange". The various examiners found slight differences in the organoleptic behaviour which, however, are not significant since they are scattered between the various series including the control bread. A differentiation between bread made of treated or untreated wheat was not possible. Since furthermore no interference was observed during baking it can be concluded that after treatment of wheat with bromophos the quality of bread is not negatively influenced.

SUMMARY: Large-scale laboratory trials on wheat treated with 8 or 12 ppm bromophos showed at temperatures usually to be found in bulk storage within a period of one year, a degradation rate of 40 - 50% after 12 months. At higher temperatures, as measured in warmer countries, the reduction of residues was more pronounced. The metabolism studies were limited to the analysis of bromoxone and 2,5-dichloro-4-bromophenol in wheat. Only the latter compound was found in an amount of less than 1 ppm.

As was seen by milling wheat bromophos penetrates into inner layers of the grains causing higher residues in the coarse bran and in the semolina bran. The flour, however, was only slightly contaminated. During the making of bread a high disappearance rate could be demonstrated resulting in residue data of about 2 ppm in bread prepared from crushed wheat after 12 months storage. Bread made with flour, the mainly consumed type, contained only traces of about 0.1 ppm of the parent compound. A disadvantageous influence on the odour or flavour of the bread which could be traced back to the treatment with bromophos was not observed. In view of the favourable toxicological properties of bromophos no harmful effects can be expected after consumption of food products containing the assayed residues.

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