Bull. Environ. Contam. Toxicol. (1996) 56:359-366

Bulletin of Environmental Contamination and Toxicology

Atrazine Movement and Dissipation in a Sandy Loam Soil under Irrigation: An Immunoenzymatic Study

S. Tasli,[1] P. Ravanel,[2] M. Tissut,[1], J. L. Thony,[3] B. Garino[4]

[1]Laboratoire de Physiologie cellulaire végétale, Université J. Fourier, BP 53, 38041 Grenoble cedex 09, France
[2]Laboratoire de Pharmacognosie, Université J. Fourier, BP 53, 38041 Grenoble cedex 09, France
[3]Laboratoire des Transferts en Hydrologie et Environnement, Université J. Fourier, BP 53, 38041 Grenoble cedex 09, France
[4]Lycée Agricole, 38260, La Côte Saint André, France

Received: 28 March 1995/Accepted: 21 August 1995

Under field conditions, the dissipation of pesticides greatly depends on the soil structure and the water movement. In fact, rain may quickly run off into streams, or pass through the soil surface, via macroporous and microporous paths, reaching the groundwater beneath more or less rapidly. Some of this water will be evaporated from the soil surface or be transpired by plants. Due to the importance of water movements in the fate of pesticides, the role of preferential movement of water and solutes through structured soils has received much attention (Thomas and Phillips, 1979, Everts et al., 1989). The fate of atrazine in soil depends on complex interactions between mass flow, diffusion, hydrodynamic dispersion, routes of water and solutes in soil, pesticide stability, sorption on soil, and metabolization by the soil microflora.

The purpose of this study was to evaluate the behavior of atrazine in soil, soil water and plants over two seasons, under field conditions, in an irrigated corn culture, in a region of intense corn culture, in the South East of France, where groundwater contamination by agrochemicals might be suspected to decrease water quality. Enzyme-linked immunosorbent assays were used in this study in order to follow the fate of this herbicide, using of a GC method as a reference.

MATERIALS AND METHODS

The experimental field, in the grounds of the Experimental Agriculture Station at La Côte Saint Andre, about 60 km west of Grenoble (Isère, France), is flat and it covers a 2 ha. area. The soil of the experimental site is shallow, sandy and rocky, and of fluvio-glacial origin. The experimental area have 8 measuring sites, each of which is equipped with a neutron probe for soil moisture measurements, associated with tensiometers and with ceramic suction cups for soil water sampling (Kengni et al. 1994). After tillage, two plots remained bare. The six other were planted with corn at the end of April. Herbicides were sprayed, pre-emergence, 2-3 days after

Correspondence to: P. Ravanel

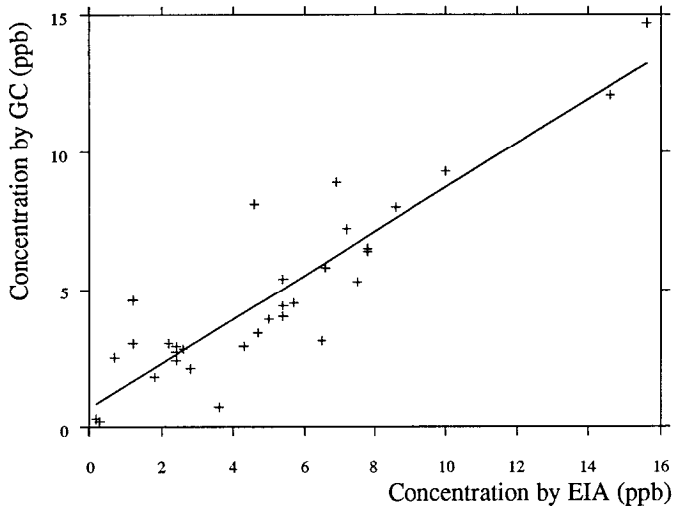

Figure 1. Linear regression of the concentration of atrazine in soil water samples (n=32) as determined by immunoassay (EIA) versus gas chromatography (GC).

sowing. Commercial formulations of the herbicides were used: atrazine (1 kg/ha) as Atraphyt EL (Sipcam-Phyteurop) and, alachlor (2 kg/ha) as Lasso (Monsanto). Each measurement site was equipped with six ceramic suction cups at three depths: two replicates at 30, 50 and 80 cm depth. Soil water samples were extracted weekly. Soil samples were collected monthly, and moisture contents were determined. Air-dried soil was sieved at 2 mm and aliquots (20 g) were extracted twice, according to the method described by Goh et al. (1991), for 1 hour with 100 ml of a methanol-water solvent (1/1 : v/v), at pH 5. Following the removal of methanol, the aqueous soil extracts were analyzed by enzyme-immunoassay (EIA) procedure. The atrazine enzyme immunoassay kits (Envirogard™ Plate Kit Triazines, ENVR P00 00) were purchased from Millipore. The wells were read using a microliter plate reader, at 450 nm (Metertech Microplate Reader Model 960, Bioblock, Illkirch, France). The GC analysis was carried out using a Hewlett-Packard 5890 instrument, equipped with a N-P detector.

RESULTS AND DISCUSSION

Figure 1 represents a linear regression analysis of the concentration of atrazine in soil water samples (n = 32) as determined by enzyme immunoassay versus gas chromatography. The results of this comparison give the following equation $Y = 0.666 + 0.805\ X$ with a correlation coefficient of $r = 0.902$. Our results are quite similar to those obtained in previous studies (Goh et al., 1991; Leavitt et al., 1991). This confirms that EIA is a useful analytical tool, giving results comparable

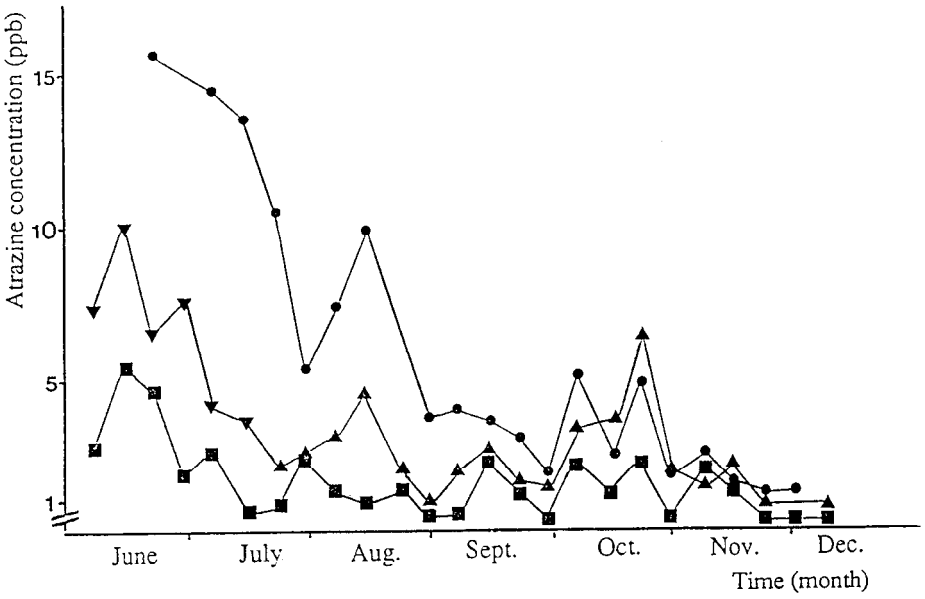

Figure 2. Changes in the atrazine concentration (ppb) in soil water sampled at 30 (●), 50 (▲) and 80 (■) cm depths during a corn culture season (1991). Treatment day: April 22, 199l.

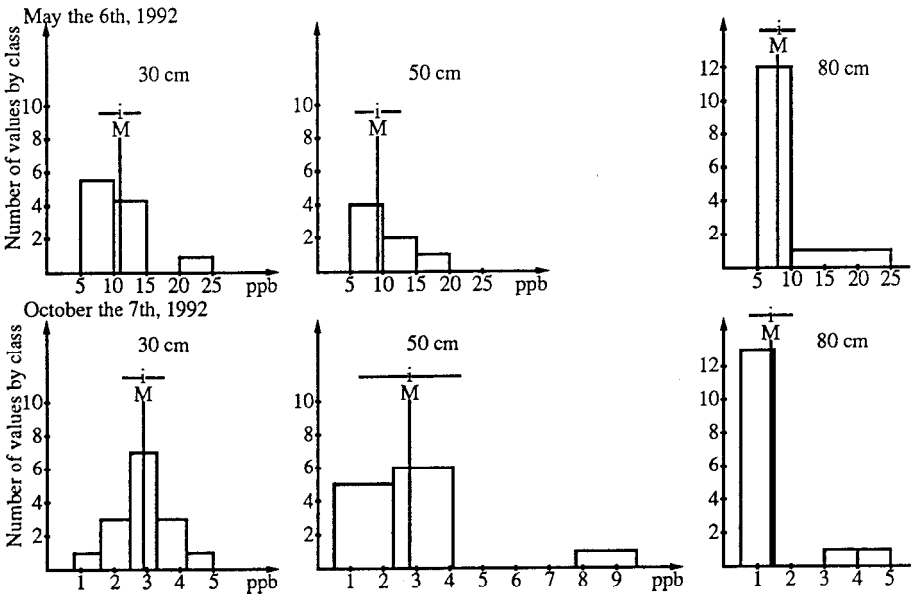

Figure 3. Frequency histogrammes for atrazine concentration in soil water. M: average value, i: confidence interval.

to the GC method for the determination of concentration of atrazine in aqueous samples. The polyclonal EIA kit used presents a possible cross-reactivity for some of the metabolites of atrazine. However, if we consider the levels of metabolites detected by the GC method in our samples, and the low sensitivity of the EIA kits for atrazine metabolites, it appears that only atrazine was actually taken into account by the EIA test used, under our experimental conditions. In the following parts of this paper, only EIA results are presented.

One of the eight plots of the experimental site was selected in 1991 for an annual assessment of the changes in atrazine content in soil water samples collected at different depths. Figure 2 shows that the annual atrazine water concentration in soil varied from 15 to 0.3 ppb. The general shape of the curves corresponded to a continuous decrease from June to December. A 60% decrease occurred between June and the end of August. Throughout the year, atrazine was present in sufficient amounts to be detected by EIA in each of the soil water samples. We can estimate that atrazine located below 80 cm, cannot be absorbed by roots or used for microbial metabolization.

From the 2 ha. experimental area, 48 water samples were collected weekly from suction cups, 12 samples for each depth. Frequency histogrammes for atrazine concentration in soil water were established (figure 3), in order to evaluate whether a regrouping of samples by date and by depth, was valid or not. The frequency histogrammes were compared with the mathematical average for the concentrations (M±), determined by measuring each sample. Figure 3 shows that, under our field conditions, regrouping of samples for each depth was an acceptable procedure. According to this regrouping procedure, we can observe that the atrazine concentration in soil water varied in the same way in 1992 as had been shown for the selected plot which was analyzed in 1991, and that a slow decrease occurred, at each depth, during the season (17 ppb to 0.3 ppb). The major difference between the two years seems to be the earliness of the decrease of atrazine concentration in 1992. Comparison of the rainfall amounts during these two years, in May and June may explain this situation (1991 : 120 mm; 1992 : 211 mm). Due to a very porous structure of our field, this observation leads us to consider the early periods of rainfall after treatment as periods of great risk for groundwater contamination.

A study of water movement inside the soil of our experimental bare sites has demonstrated that drainage taking place to a one-meter depth represented 50% of the rainfall amounts between April and the end of September, and 90% between October and March (Kengni et al., 1994). In contrast, on sites with corn culture, the cumulative precipitation amounts received by soil under both rainfall and irrigation during the dry July-August periods were not followed by drainage (Kengni et al., 1994). According to these values, the amounts of atrazine leached from the shallow one-meter thick soil layer over one year can be estimated (Table I). This leaching estimate was performed monthly, taking into account : a)- the amount of water accumulated monthly through rain and irrigation, b)- the % of drainage under the crop for each month (50, 0 or 90% depending on the period), c)- the monthly average concentration of atrazine in soil water at 80 cm depth. This leaching represented 0.7% of the applied treatment during 1991, and 0.3% during 1992. However, even if these values are greater than those obtained by Muir and

Table 1. Estimated atrazine amounts leached from the shallow one-meter thick soil layer over one year.

Month	Rain and irrigation (mm)	Monthly mean concentration in soil water at 80 cm (ppb)	Drainage[a] (mm)	Estimated atrazine leaching (g/ha.)
(1991)				
May	26.4	4.6	13.2	0.6
June	92,8	4.6	46.4	2.13
July	15.6	1.6	0	0
August	91.9	0.95	0	0
September	135.2	1.70	67.6	1.15
October	133.6	1.55	120	1.86
November	55.9	0.90	50.4	0.16
Jan-April	170.2	0.20	153	0.30
				TOTAL 6.65 g/ha
(1992)				
May	92.9	2	46.5	0.93
June	118.3	0.8	59	0.47
July	133.4	0.56	0	0
August	149.3	0.9	0	0
September	81.2	0.5	41	0.2
October	159.2	0.35	143	0.5
November	98.2	0.25	88.4	0.22
December	64.2	0.2	58	0.12
Jan-April	120.3	0.2	108.2	0.2
				TOTAL 2.64 g/ha

[a] Drainage under crop: May-June and September: 50% of the total rain amount, July-August 0% of the total rain + irrigations, October to April: 90% of the total rain amount (Kengni et al., 1994).

Baker (1976), and by Southwick et al. (1990), this leaching is probably greatly underestimated. One fact can illustrate this point. Two days after the 1994 treatment, an abundant rainfall (45 mm) occurred in a short period of time (3 hours). During this rain, different samples of soil were collected. The supernatant fraction in the upper layer of soil (0-30 cm), quickly removed from the samples, presented an atrazine concentration close to 1500 µg//L. Two days later, at a 30 cm depth, in the water obtained from suction porous cups the atrazine concentration was 19.2 µg/L. Such a concentration was 75 fold lower than that obtained during the high rainfall. Fifty % of the rainfall amounts being drained during this period (i.e. 225 m^3/ha), an atrazine concentration of 1500 µg/L in soil water represents the possibility of a large quantity of atrazine (350 g/ha) leaving the upper layers of soil.

Atrazine is a pre-emergence herbicide which acts inside the leaves, on the D_1 protein located in the thylakoid membrane inside the chloroplasts. The a.i. follows a water flow which take place between the soil water and the atmosphere via root hairs, xylem vessels and leaves. The concentration of a.i. in topsoil water (0-10 cm) must be sufficiently high during 2-3 months, to ensure the inhibition of all the

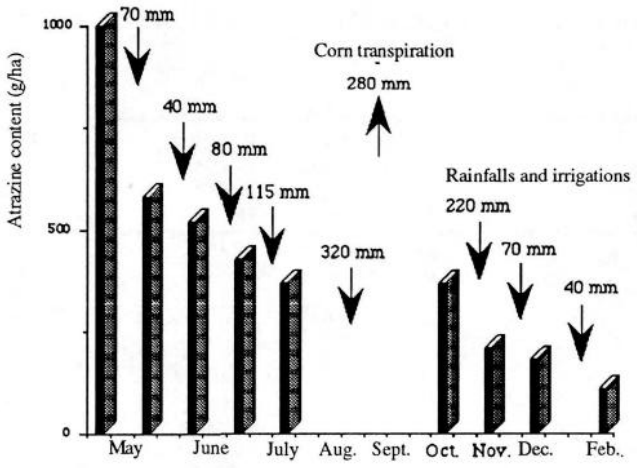

Figure 4. Changes in the amounts of atrazine present in the 40-cm shallow thick layer of soil (1992). Numbers on the arrows represent the rainfall and transpiration amounts of water (mm)

targets continuously biosynthesized at the level of new growing leaves of different species of weed seedlings sprouting at different periods of time. We named such a concentration of a.i. "the critical concentration". To kill a plantlet, all its D_1 proteins must be inhibited for a sufficiently long period of time. To ensure a persistent herbicidal effect, 1 kg/ha of formulated atrazine is required under our field conditions. This quantity is that which is able to maintain a critical concentration of a.i. in the water, enough to kill the weeds, for a sufficiently long period of time. We have demonstrated, under laboratory conditions (results not shown), that this critical concentration of atrazine must reach 200 µg/L in order to kill the weeds. This concentration is 2000 times the highest concentration acceptable in Europe for one pesticide in drinking water (0.1 µg/L for individual pesticides). If the critical concentration is still maintained in the topsoil water 2 or 3 months after treatment to ensure a phytotoxic effect, it signifies that just after treatment, during wet periods, the topsoil water is probably greatly upper than the critical concentration. During the early period following treatment, if abundant rains occur, peaks of a. i. in the drained water may occur which considerably exceed European standards for the potability of drinking water.

Herbicide treatment was performed on the 23rd of April 1992 (atrazine: 1 kg/ha). At this stage, the amount of atrazine arising from the previous treatment (1991), in the shallow 40 cm-thick soil layer, was close to 50 g/ha. So, just after treatment, the stock in this layer represented approximately 1 kg/ha. This amount decreased rapidly during May (figure 4). Practically 50% of the a.i. applied had disappeared from the 40 cm deep layer 30-45 days after treatment, 25% remaining in the first 10 cm and only 10% in the 0-3 cm thick layer which is considered to be the zone where the presence of a. i. is required in order for it to play its herbicidal role. In the middle of December, 18% of the total amount applied remained in the 40 cm-thick soil layer.

Three factors may explain the 50% decrease in soil atrazine content, one month after treatment 1) volatilization, which was observed to reach 15% of the a.i. applied, under our field conditions, in 1992 during the first days after treatment; 2) metabolization by the soil microflora, greatly favoured by a warm monthly temperature (average in May, for air : 15.8 °C) and optimal moisture ; 3) a large underestimation of the leached amounts due to an inadequate soil water sampling system especially during spring, when large-scale rainfall occurred.

An estimation of the soil content during February 1993 was carried out after measurement of the atrazine amounts present in each 10 cm thick layer, down to a one meter depth. These amounts, layer by layer, from the top to the one-meter depth, were: 46.6, 30.3, 26.7, 10, 13.3, 6.7, 10, 2.9, 1.3 and 1 g/ha, respectively. So, 285 days after treatment, 15% of the applied amount was still present in the one-meter layer, and 75% of this amount, was located in the upper 40 cm, where there was a high organic matter content (2.6%). Due to the presence of 150 g/ha at the beginning of February it appeared unlikely that a total disappearance of atrazine should occur before the new treatment at the end of April 1993.

Corn growth requires a large amount of soil water, as a consequence, atrazine dissolved in this water is absorbed by the corn. Corn detoxifies the a.i. by metabolism, metabolites being physiologically inactive with respect to the photosynthetic process. We have taken into account 1) the water requirements per ha., for corn between the beginning of June and the middle of September, when transpiration is highest, and, 2) the highest concentrations of atrazine measured in our samples of soil water. These measurements led us to an estimation of the atrazine amounts absorbed by the culture. The estimate amounts of atrazine absorbed by corn, by periods of 15 days, between June and the middle of September were: 0.75, 2.8, 3.6, 2.4, 2.9 and 2.4 g/ha, respectively. The highest value corresponding to the atrazine absorbed during the season was estimated to be close to 15 g/ha, the water transpired representing 87% of the total water added to the soil during the period studied (Figure 4).

As a whole, it appears that immunoenzymatic assays can give an accurate measurement of the atrazine concentration of water and soil samples taken from field stations. Under our experimental field conditions, the volatilization process was estimated to represent 15% of the amount sprayed in April. The amounts of atrazine absorbed by plants (essentially by the corn) represented 1.5%, whereas 10% was still present in the one-meter thick layer, one year after treatment (75% of this amount being located in the upper layer: O-30 cm). The total average amount estimated in this way is therefore close to 250 g/ha/year. One kg/ha is sprayed on the soil at the beginning of period of each corn culture, thus 750 g/ha is consequently absent from our evaluation. For the most part, this amount corresponded to soil metabolization (Tasli et al., submitted). In fact in the upper soil layer (0 to 30 cm) a part of the atrazine was quickly transformed into metabolites (deethylatrazine, deisopropylatrazine, hydoxylated metabolites ...) not studied here. Under controlled conditions, we have verified that a very intense soil metabolization of uniformly ^{14}C labelled atrazine occurred: 45 days after the beginning of the incubation experiment, 40% of the labelled carbon was in the form

CO_2. However, it should be noted that our system of weekly water sampling underestimates leaching: transfer of solubilized atrazine through the upper layer of soil is probably a very rapid phenomenon during abundant rainfall, especially during the first month after treatment, as shown by water and atrazine transfer studies on soil columns and lysimeters (results not shown).

For all the above reasons, our experimental station seems to present a type of soil which has a high risk of groundwater stock contamination during periods of rain occurring just after treatment. Concerning atrazine, the major risk involves the possible rapid transfer, via macropores, of the formulated a.i. part, which is not associated with the microporous soil fraction. As far as metabolites transfer is concerned, the situation appears to be quite different, and the hazard may be situated during other periods of the year, because the appearence of these metabolites needs a period of atrazine-micropore interaction (microflora) in order to insure metabolization, and, later, metabolites must leave the microporous fraction in order to reach the macroporous stream and the aquifer.

Acknowledgments. The authors of this study are greatful to the French Ministry of Agriculture for financial support and especially our correspondant, Eric Dabene.

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