N,N-diethyl-m-toluamide transformation in river water

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P. Calza [1,2]*, C. Medana[2], E. Raso[2], V. Giancotti[2], C. Minero [1,2]

The paper deals with the aqueous environmental fate of N,N-diethyl-m-toluamide (DEET), one of the most widespread and efficient mosquito repellents. The investigation involved monitoring of the DEET decomposition and the identification of intermediate compounds.

Initially, control experiments in the dark and under illumination were performed on sterilized and river water spiked with DEET, with the aim to simulate all possible transformation processes occurring in aquatic system. Under illumination, DEET was degraded and transformed into numerous organic intermediate compounds, 37 of which could be identified. Several isomeric species were formed and characterized by analyzing MS and MS^n spectra, and by comparison with parent molecule fragmentation pathways.

These laboratory simulation experiments were verified in the field to check the mechanism previously supposed. River water was sampled and analysed at eight sampling points. Among the transformation products (TPs) identified in river water spiked with DEET, twelve of them were also found in natural river water. The transformation occurring in aquatic systems involved dealkylation, mono- and poly-hydroxylation followed by oxidation of the hydroxyl groups and cleavage of the alkyl chains. Two TPs were principally formed in dark condition, while the others are mainly produced through indirect photolysis processes mediated by natural photosensitizers.

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Keywords: HRMS; DEET; unknown transformation products; river water, photochemistry

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INTRODUCTION

This study enlightens the fate of N,N-diethyl-m-toluamide (DEET), one of the most widespread and efficient mosquito repellents, in the aquatic environment. DEET is included in Pharmaceuticals and Personal Care Products (PPCPs) that can be considered to be emerging contaminants of environmental concern because of their continuous release into the aquatic environment, their persistence and evidences of ecotoxicological effects (Cunningham et al., 2006).

DEET permits a total protection against mosquitoes bite for several hours (Fradin et al., 2002) and is known to be persistent (Costanzo et al., 2007) but weakly toxic toward fishes, birds and invertebrates (Pietrogrande et al., 2007). The majority of DEET enters waterways via sewage effluent following washing off and absorption/excretion by humans; almost 20% is adsorbed through skin, metabolized or excreted (Sudakin et al., 2003). Even if DEET can be efficiently abated through advanced oxidation processes (AOPS) methods such as Fenton (Zhang et al., 2006; Zhang et al., 2007) and ozone treatment (Snyder et al., 2006), it was found at trace level in surface waters, groundwater and waters for human consumption (Sandstrom et al., 2005; Kolpin et al., 2004; Langford et al., 2008; Costanzo et al., 2007). DEET presence was detected in USA rivers, at an average concentration of 0.05 μg/L (Sandstrom et al., 2005); into 97% of surface waters in Australian Eastern coast at an average concentration of 0.093 μ g/L (Costanzo et al., 2007); in Europe, across river Rhine at concentration ranging from 15 to 30 ng/L (Schwarzbauer et al., 2005 Quednow and Püttmann, 2009), across Norway coast at concentrations 0.4-13 ng/L (Langford et al., 2008; Weigel et al., 2004), and in the North Sea, where it is considered as a ubiquitous pollutant with an average concentration of 1.1 ng/L in summertime (Weigel et al. 2002).

A previous study was concerned with the mechanism of TiO_2-mediated photolysis of N,N-diethyl-m-toluamide (DEET) (Medana et al., 2011); the photocatalytic process can be used to artificially produce degradation compounds similar to those formed in oxido/reductive metabolic and environmental pathways (Calza et al., 2010; Calza et al., 2004). A total of 51 unknown DEET

degradation products were identified and characterized by multiple stage mass spectrometry, as described previously (Medana et al., 2011).

In this study, we have focused on the DEET fate in Po River waters (North Italy). The goal was attained in two steps. Firstly, laboratory experiments were performed by using river water spiked with DEET under dark or simulated solar light. Secondly, all the possible main and secondary transformation products (TPs) were searched in natural samples; DEET and all identified TPs were monitored in eight samples collected from the Po River. Within this framework, the fate of DEET in the river water environment is clarified.

2. Experimental

2.1. Materials and reagents

DEET (97%, solubility in water 1g/l), isoxsuprine hydrochloride, sodium nitrite, sodium nitrate, phenol, catechol, resorcinol and hydroquinone were from Sigma-Aldrich (Milan, Italy). Formic acid (99%) was from Merck (Milan, Italy). HPLC grade water was from MilliQ System Academic (Millipore, Milan, Italy). HPLC grade acetonitrile (BDH) was filtered through a 0.45 μm filter before use.

2.2. Irradiation procedures

Sterilized water or Po river water sample were spiked with DEET (15 mg L^{-1}) and irradiated. Irradiations were performed using a Philips (Monza, Italy) TLK/05 lamp (40 W/m^2) with the maximum emission at 360 nm. Irradiation experiments were carried out in Pyrex glass cells containing DEET. The temperature reached during the irradiation was 38 ± 2°C.

2.3. Sample Preparation

Po river water samples were collected in a sampling campaign performed from 1 to 4 July 2008 along the whole Po river tract. The map showing the sampling points is reported in Fig S1 as supplementary material, while chemical-physical features of the samples were shown elsewhere (Calza et al., 2010). Samples were collected 2 m far from the river border using brown glass bottles, kept in the dark and promptly analysed. Field blanks were also prepared in each sampling site using Sample 1 water to ensure samples were not contaminated with DEET.

Samples were then concentrated on SPE cartridge and analyzed by HPLC/HRMS. For solid phase extraction (SPE) Strata X (Phenomenex, Casalecchio di Reno, BO, Italy) cartridges were used. Water samples (200 mL) were spiked with 200 µL isoxsuprine (1 mg/L) used as recovery standard. Elution was done with 2 mL CH_3OH, 2 ml of 2 % ammonia in CH_3OH. Eluted solutions were dried under nitrogen flux and then reconstituted with 200 µL 0.05% formic acid and directly injected into HPLC/MS. Quantitative data were obtained through an external calibration after normalization on isoxsuprine signal. For DEET, limit of detection (LOD) after concentration on SPE cartridges is 0.5 ng/L. The same extractive procedure was applied as blank analysis to an ultrapure water sample spiked with DEET and subjected to illumination. With this method, all formed TPs showed a recovery percentage >90%.

2.4. Analytical procedures

2.4.1. Liquid chromatography

Chromatographic separation followed by MS analysis was run on a Phenomenex (Casalecchio di Reno, BO, Italy) Synergi C18 column, 150 × 2.0 mm using an Ultimate 3000 HPLC instrument

(Dionex, Milan, Italy). Injection volume was 20 μL and flow rate 200 μL/min. Gradient mobile phase composition was adopted: 5/95 to 100/0 in 21 min acetonitrile/ammonium acetate 0.1 mM.

2.4.2. Mass Spectrometry

An LTQ Orbitrap mass spectrometer (ThermoFisher, Rodano, Italy) equipped with an atmospheric pressure interface and an ESI ion source was used. The LC column effluent was delivered into the ion source using nitrogen as sheath and auxiliary gas. The source voltage was set to 4.1 kV. The heated capillary temperature was maintained at 265°C. The acquisition method used had previously been optimized in the tuning sections for the parent compound (capillary, magnetic lenses and collimating octapoles voltages) in order to achieve maximum sensitivity. The tuning parameters adopted for the ESI source were: capillary voltage 7.00 V, tube lens 44 V; for ions optics: multipole 00 offset -1.25 V, lens 0 voltage -4.00 V, multipole 0 offset - 4.75 V, lens 1 voltage -13.00 V, gate lens voltage -52.00 V, multipole 1 offset -15.00 V, front lens voltage -5.00 V. Mass accuracy of recorded ions (vs. calculated) was ± 1 millimass unit (mmu) (without internal calibration).

3. Results and Discussion

DEET transformation through a photooxido/reductive pathways using TiO_2 as photocatalyst occurs through the formation of numerous TPs (Medana et al., 2011), as summarized in Table 1 as supplementary material. All of these compounds could be similarly formed in aquatic systems and consequently their presence was monitored firstly in river waters sampled in Turin on 04 July 2008 and enriched with DEET just after sampling and then in natural Po river water.

3.1. Transformation of DEET in River Water

3.1.1. Laboratory Simulation

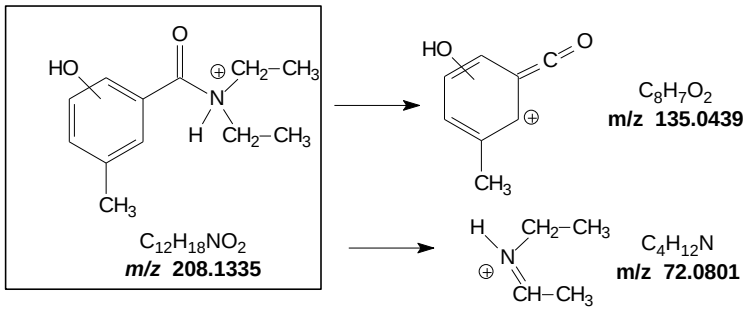
Measures in the dark and by direct photolysis on sterilized water showed that photolysis and thermal decomposition did not contribute to DEET decomposition.

Dark experiments in river water proved that DEET disappearance slowly occurred ($t_{1/2}$ 15 days, see Figure 1). In the considered times (20 days) several TPs were formed at a detectable concentration as reported in Figure 2. The formation of the species at *m/z* 180-A, 180-B, 222-A, 178-A, 178-B, 164 and of four isomeric species at *m/z* 208 occurred in the dark. Two isobaric species at *m/z* 178 were evidenced holding different nominal mass values; the first one at *m/z* 178.1228 ($C_{11}H_{16}ON$), namely 178-A can be attributed to the structure previously described when using TiO_2 (Medana et al 2011), while the second one at *m/z* 178.0882 ($C_{10}H_{12}O_2N$), namely 178-B, is well matched with the formation of *N*-ethyl-*m*-formylbenzamide, already recognized in metabolic studies (Constantino and Iley, 1999). All of the other identified species well matched with the compounds formed through a TiO_2 photocatalytic process as collected in Table 1. Among these TPs, the compounds 178-A and at *m/z* 164 (Fig. 2, left) together with TP 222-A (Fig. 2, right) are quickly formed at an high amount, so being the main compounds produced through biotic route. In agreement with biotic data available in literature (Seo et al., 2005, Constantino and Iley, 1999), micro-organisms induce monohydroxylation, demethylation on the aromatic ring and detachment of the alkyl chain.

Under illumination, DEET transformation in river water easily occurred ($t_{1/2}$ decreases from 15 to 5 days) and proceeded through the formation of the TPs plotted in Figure 3. In addition to the former described compounds, other TPs are easily formed at high amount as shown in Figure 3A and 3B, namely hydroxylated and/or oxidised derivatives. In addition, numerous TPs are formed in a lesser extent with a slightly delayed time, all collected in Figure 3C-F. Most of them coincide with those formed through the TiO_2 photo-induced process (see Table 1). The main transformation products were isomeric hydroxy-DEET (*m/z* 208), N,N-diethyl-m-benzenamide and *N*-ethyl-*m*-formylbenzamide (*m/z* 178A and B) and N-ethyl-m-toluenamide (*m/z* 164). Among the 51 TPs previously characterized (Medana et al., 2011), 35 were also found in river water samples. In

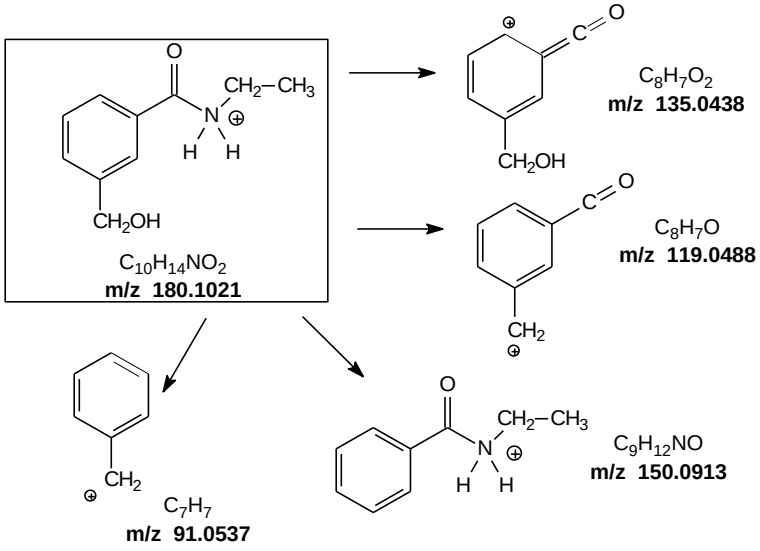
addition, two new isomeric forms for the species at *m/z* 208 and 180 were identified in river water due to an additional transformation route.

The new compound at *m/z* 208.1335 (labelled **208-E**) fragmented to the product ions summarized in Table S1 in supplementary material. MS^2 spectrum shows the product ions at *m/z* **72.0801** and **135.0438**; the first one permits to exclude an hydroxylation on the alkyl chain, while the second one suggests the addition of an OH group on the aromatic ring, as shown in Scheme 1.



Scheme 1. Fragmentation pathway followed by the species 208-E

Looking closer to the species at *m/z* 180.1021, a new isomeric form was detected (labelled **180-E**), and its temporal profile during irradiation is reported in Figure 3. Main MS^2 product ions are collected in Table S1 and Scheme 2.



Scheme 2. Fragmentation pathway followed by the species 180-E

The formation of a product ion at *m/z* **135.0438** suggests the presence of the alcoholic group on the aromatic ring; the formation of a product ion at *m/z* **150.0913** through formaldehyde loss is well-matched with the proposed structure (see Scheme 2).

Figure 4 reports the maximum amount for each TP detected during DEET decomposition in the different laboratory simulations (river water under dark (D) or light induced experiments (L)). By comparing D and L river water data, even if the time evolution profiles are similar in both cases (see Figures 2 and 3), it is clearly seen that light induces the formation of a larger number of TPs and at greater amount when those compounds are formed in both experiments, but with two exceptions. The species at *m/z* 164 and 178-A are formed in an even higher amount in dark experiments, as a proof of their favoured formation by biotic process.

3.1.2. In Field Transformations

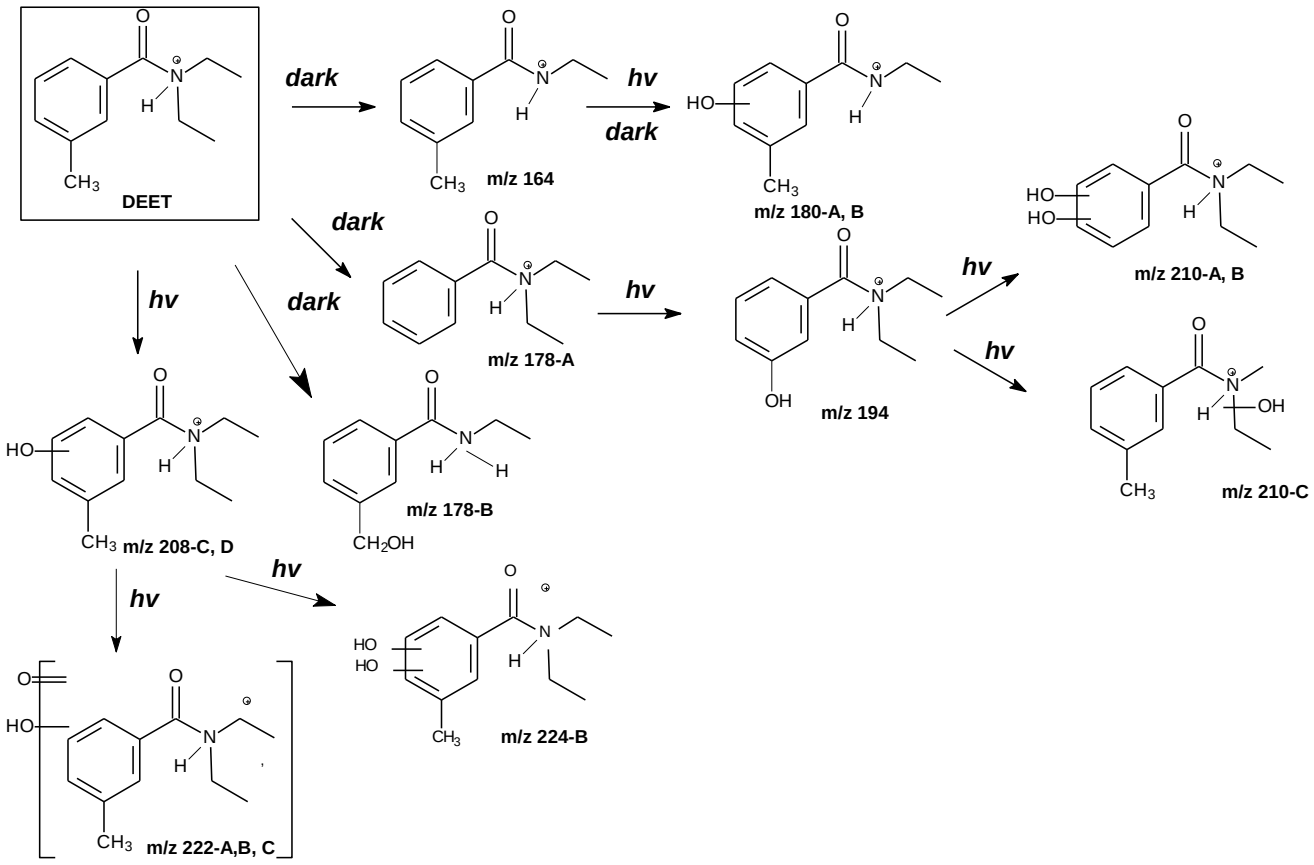
DEET and its transformation products were searched out in all Po river samples (see Table 1 and Figure 5). Sample 1 was collected close to the river source and neither DEET nor its transformation products were detected. Sample 1 water was then used as field blank matrix. Conversely, DEET was found in all other samples, at concentrations collected in Table 2, reaching maxima in samples 3 (Turin, 137 ng/L) and 6 (Casalmaggiore (CR), 155 ng/L). Few articles are available about emerging contaminant presence in Po River water, all focused mainly in drugs detection (Zuccato et al., 2000; Zuccato et al. 2006, Castiglioni et al., 2004), while there are no data on DEET monitoring in Italian river waters. These high concentrations are not surprising if it is considered that Po river is in a region characterized by a long and hot summer, rendering it a good place for mosquito growth. For such, DEET in summertime is widely used and, following washing off and absorption/excretion by humans, it could easily reach wastewater and diffuse in the aquatic system. It was also observed that DEET is persistent and could then diffuse across a long distance from the intake point.

In addition to DEET, several species identified in laboratory experiments were also detected in natural Po river water, as displayed on Figure 5, and their formation probably occurred though a combination of biotic and abiotic pathways; all the recognized TPs may be linked through the transformation pathways summarized in Scheme 3.

TPs at *m/z* 222 (hydroxylation and oxidation), 164 (detachment of the alkyl chain) and their hydroxyderivatives 180-A and 180-B and at *m/z* 178 (demethylation of the benzenic ring or dealkylation/hydroxylation) were formed as main products. Due to the discussion above, TPs 164 and 178-A should be mainly formed from biotic processes, while the formation of TPs at *m/z* 180-A, 210-A, 210-B, 210-C, 222-A, 222-B and 222-C has to be chiefly ascribed to abiotic routes. It is worth noting that these compounds coincide with the TPs formed at a higher rate (and amount) in the laboratory experiments performed on river water spiked with DEET. Furthermore, they were also formed during photocatalytic experiments, but not by direct photolysis (Medana et al., 2011), so that their formation in the Po River samples could be attributed to indirect photolysis processes mediated by natural species such as dissolved organic matter, nitrite and nitrate ions, H_2O_2 and iron species (Boule et al., 2005).

Even if in the laboratory experiments the compounds at *m/z* 208 (monohydroxylation) was among the main species, in natural water samples it is only detected at low concentration in the Ferrara sample (sample 7) in two isomeric forms (C and D).

The transformation products 210-A, B and C (the demethylated and bihydroxylated species), were detectable only in sample 2 (Moncalieri, TO), that, together with sample 8 (Porto Tolle, RO), was the richer in transformation products.



Scheme 3. Proposed DEET's transformation pathways followed in Po River water.

TPs formed at low amount in the laboratory simulation were not detected in the natural samples, even if their evolution profiles are compatible with these followed by the detected species, so suggesting their formation below the detection limit.

4. CONCLUSIONS

DEET was detected in all river samples, together with a number of degradation products previously characterized through a TiO_2 photocatalytic process coupled with HPLC/HRMS analysis, allowing the monitoring of drug presence and transformation in environmental samples.

Fifteen TPs were identified in Po river waters and four different transformation routes were proposed. Some of them seem to be mainly formed through a biotic process (178-A and B, 164),

while other TP_S formation proceeds through only indirect photolysis process (i.e. 210-A, 210-B, 210-C, 222-B, 222-C). This approach has permitted not only to assess the DEET presence in natural waters, but also to identify the transformation routes recognized in simulative experiments, also occurred in the aquatic environment.

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Figure captions

Figure 1. Disappearance of DEET (15 mgL^{-1}) in the dark and under illumination in Po river water.

Figure 2. TPs formed in Po river through dark conditions. *(left)* species at *m/z* 208 (refer to left axis scale), 178 and 164 (referred to right axis scale), *(right)* species at *m/z* 180 (A and B) and 222-A**.**

Figure 3. (A-B) Main TPs (reported as MH^+) formed from DEET in Po River under illumination as a function of irradiation time. (C-F) Secondary TPs formed from DEET in Po River under illumination as a function of irradiation time.

Figure 4. Comparison among maxima TPs (reported as MH^+) concentration formed in the dark and under illumination in Po river water.

Figure 5. TPs profiles (reported as $M+H^+$) of concentration across the River (1. Crissolo (CN), 2. Moncalieri (TO), 3. Torino, 4. S. Raffaele Cimena (TO), 5. Spessa (PV), 6. Casalmaggiore (CR), 7. Ferrara, 8. Porto Tolle (RO)).

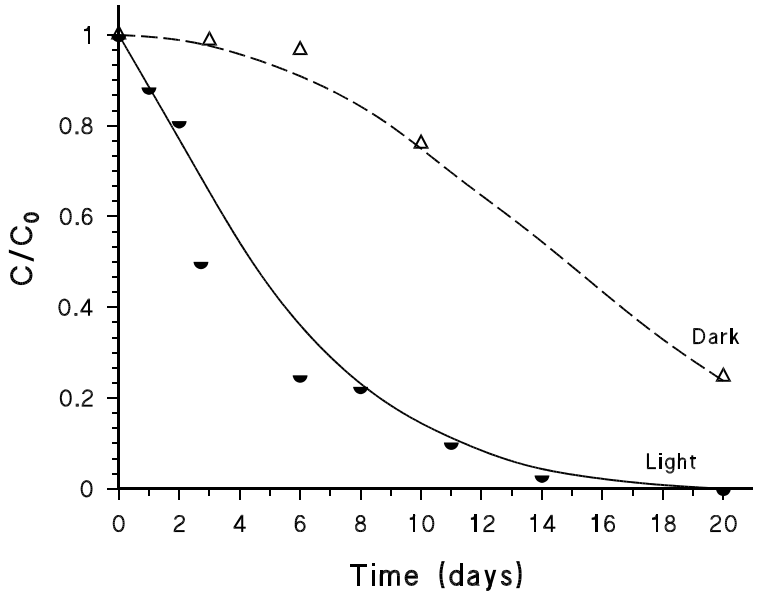


Figure 1

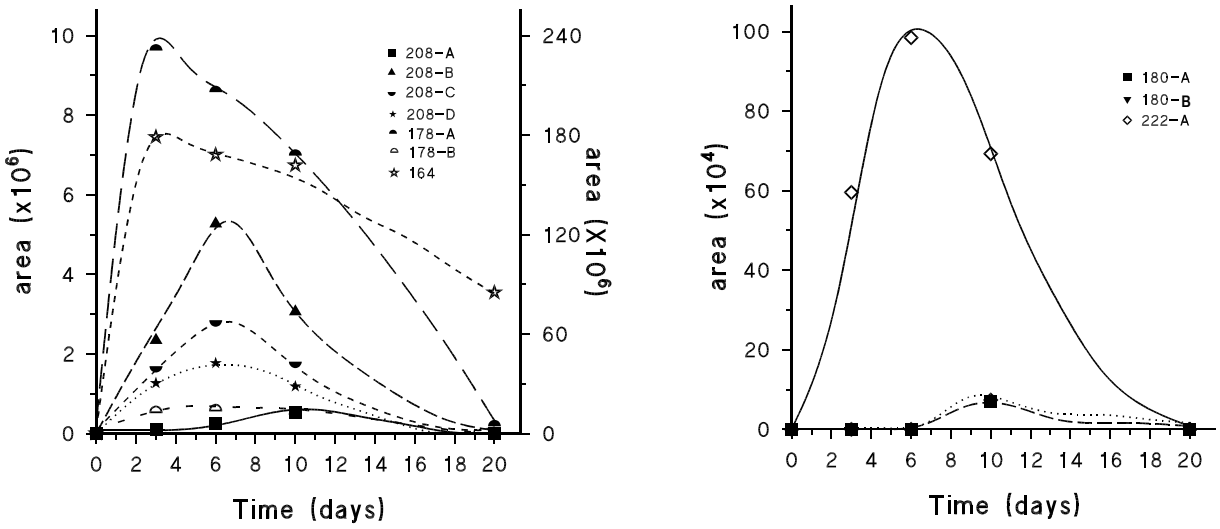

area (x10^6)
area (X10^6)
Time (days)
208-A
208-B
208-C
208-D
178-A
178-B
164
area (x10^4)
Time (days)
180-A
180-B
222-A

Figure 2

Figure 3

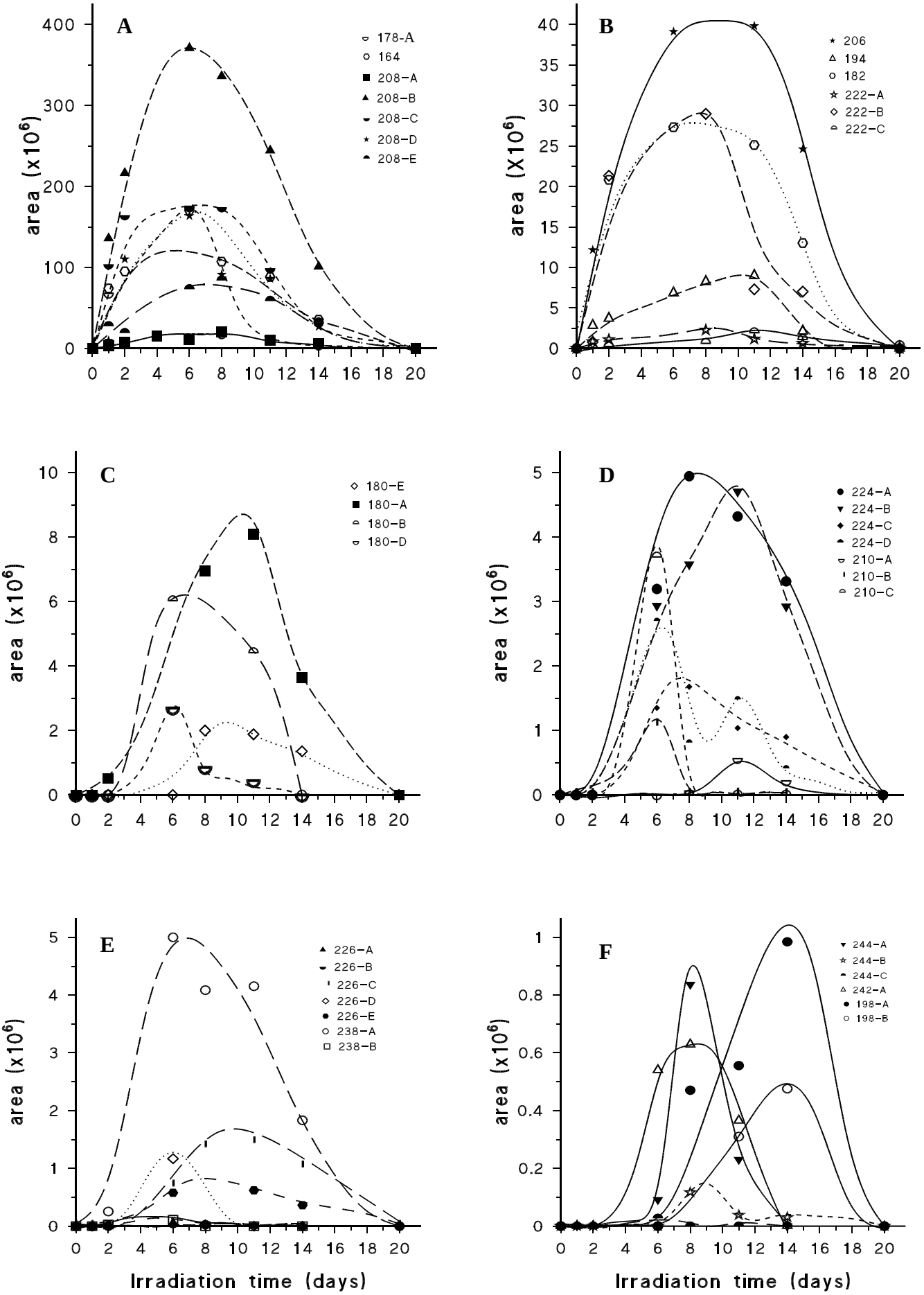
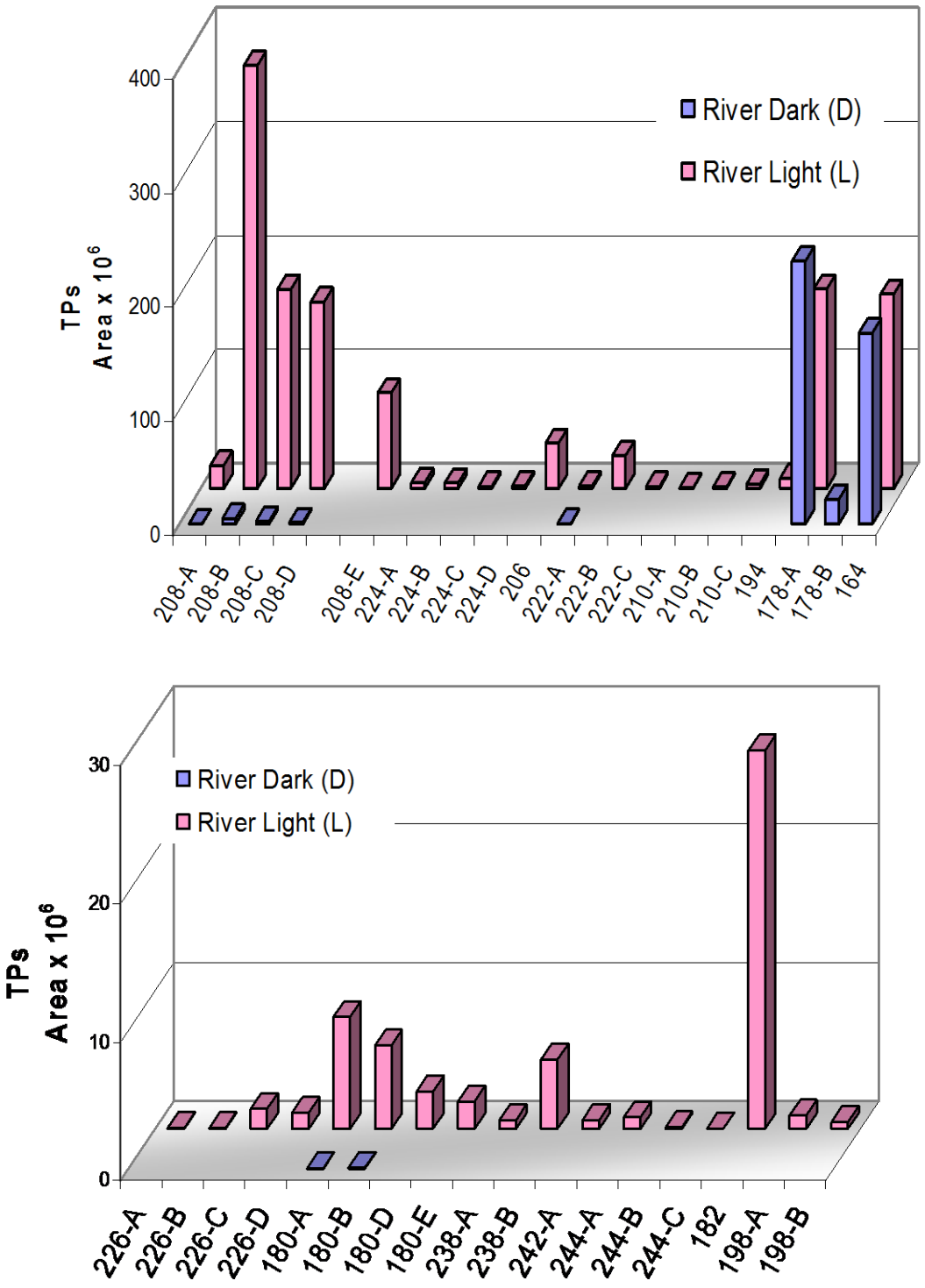

A
area (x10^6)
178-A
164
208-A
208-B
208-C
208-D
208-E
B
area (X10^6)
206
194
182
222-A
222-B
222-C
C
area (x10^6)
180-E
180-A
180-B
180-D
D
area (x10^6)
224-A
224-B
224-C
224-D
210-A
210-B
210-C
E
area (x10^6)
226-A
226-B
226-C
226-D
226-E
238-A
238-B
Irradiation time (days)
F
area (x10^6)
244-A
244-B
244-C
242-A
198-A
198-B
Irradiation time (days)

Figure 4


TPs
Area x 10^6
400
300
200
100
0
River Dark (D)
River Light (L)
208-A
208-B
208-C
208-D
208-E
224-A
224-B
224-C
224-D
206
222-A
222-B
222-C
210-A
210-B
210-C
194
178-A
178-B
164
TPs
Area x 10^6
30
20
10
0
River Dark (D)
River Light (L)
226-A
226-B
226-C
226-D
180-A
180-B
180-D
180-E
238-A
238-B
242-A
244-A
244-B
244-C
182
198-A
198-B

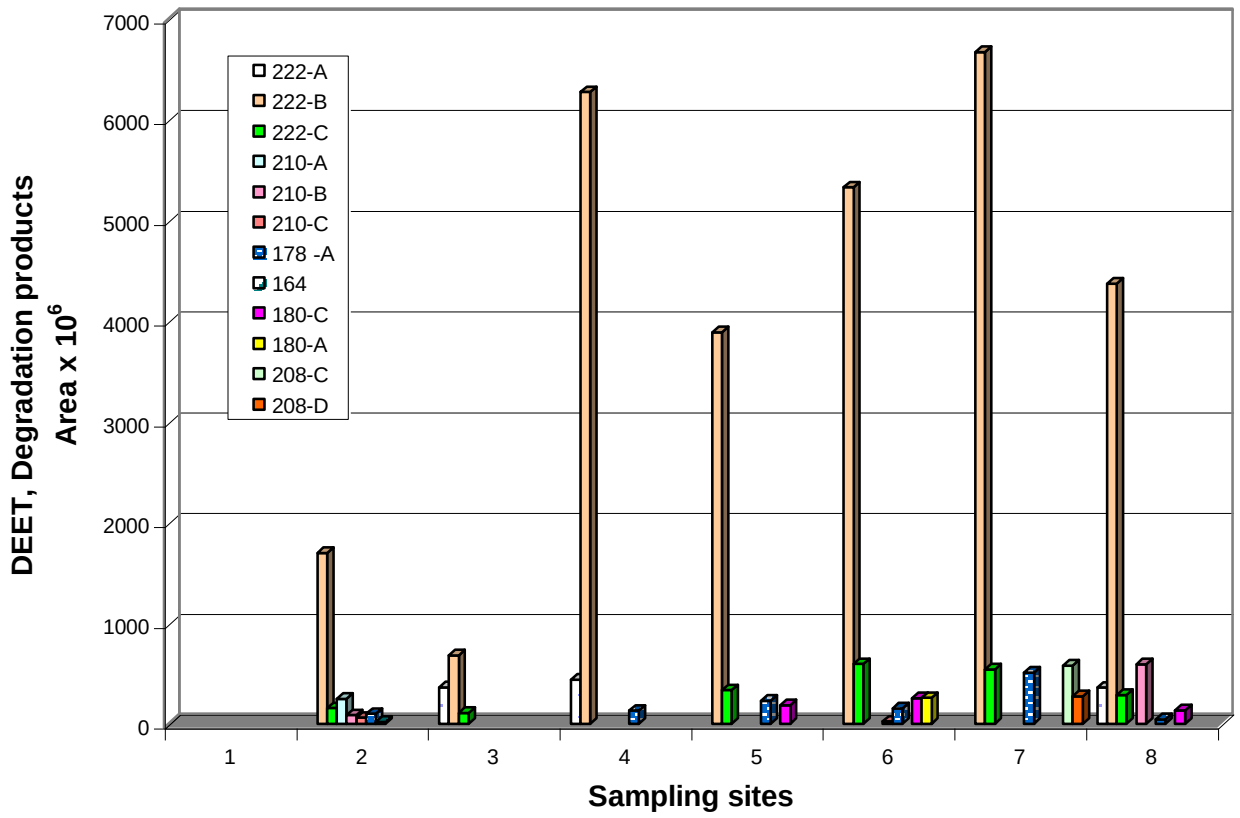

DEET, Degradation products
Area x 10^6
7000
6000
5000
4000
3000
2000
1000
0
222-A
222-B
222-C
210-A
210-B
210-C
178 -A
164
180-C
180-A
208-C
208-D
1
2
3
4
5
6
7
8
Sampling sites

Figure 5