Ecotoxicity and biodegradability in soil and aqueous media of lubricants used in forestry applications

Christine Cecutti *, Dominique Agius

Laboratoire de Chimie Agro-Industrielle – UMR 1010 INRA/INPT-ENSIACET, 118, route de Narbonne, 31077 Toulouse, Cedex 04, France

ARTICLE INFO

Keywords:
Biodegradability
Ecotoxicity
Soil
Vegetable based oils
Lubricants

ABSTRACT

The work presented in this article focuses on the environmental impact of hydraulic fluids used in forestry. Migration and biodegradability of three biolubricants and a mineral lubricant were monitored in two forest soils and in a liquid medium. These studies proved that biolubricants were easily degradable products and showed ultimate biodegradability rates significantly higher than those of the fluid of mineral origin, specially in a soil environment. This superiority was even greater when fluid behaviour was observed after 1000 h of use. Ecotoxicity test enabled the classification and comparison of biolubricants and showed that toxicity levels of the biolubricants were never high however, even after use, as compared to petroleum-based fluid.

1. Introduction

The aquatic environments, that is either fresh or sea water environments, are also the centre of many natural biological processes and special attention was therefore given to them. By way of proof, numerous tests were developed to observe the behaviour of the substances in these environments. The ecotoxicity tests used today as reference are OECD Guidelines 201, 202 and 203 (OECD, 1984) which assess the environmental impact of a substance in an aqueous environment by studying its impact on algae, daphnia and fish, standards species which representing the aquatic compartment. Recognised biodegradability tests, largely commented in the literature (Battersby, 2000; Willing, 2001; Eisentraeger et al., 2002; Pagga, 1997; Haus et al., 2001), such as the OECD 301 series (OECD, 1992) or CEC L-33-A-93 test (CEC, 1995) are available to assess biodegradability in water. These various methods make it possible to assess the impact of chemicals on the aquatic environments (Garcia et al., 2007; Haus et al., 2003).

Special attention however should be given to soil, as it is as complex an environment and essential to life as water and the centre of great biological activity. Besides the biodegradation and ecotoxicity parameters, the assessment of the environmental impact of a substance deposited on the ground should also take into account the migration parameter through the various soil layers. When a product is deposited on the soil, it should firstly be determined whether it is carried along by surface water to nearby streams or if it penetrates the ground; in the first case it will be necessary to study its behaviour in an aquatic environment. Or if the compound penetrates the ground, monitoring will be required to survey its degradation in time and in space: a too rapid migration of the chemical along with drainage waters will lead to the pollution of the ground waters and the water table. On the contrary, if the chemical remains adsorbed by the soil particles, as long as it is biodegradable, it will be transformed by the soil's microorganisms and cause no environmental damage. The importance of determining both the migration properties of the chemical substances and their biodegradability in the soil is underlined here. Monitoring the substances in the soil through space and time may provide the required information. Soil columns or lysimeters are efficient tools for this purpose. Many studies were performed in a lysimeter to monitor the evolution of pesticides and also compounds derived from vegetable materials in the soil (Agius et al., 1999; Cecutti et al., 2002; Cecutti, 2003). The purpose of these studies was to measure the primary biodegradability corresponding to the disappearance of the parent compound.

Experiments in lysimeters are however relatively complex and costly to implement. To assess a substance's biodegradability simpler laboratory tests are therefore favoured like OECD 301 guidelines. Whereas such tests are widely used in aqueous environments, they are however less frequently carried out in soil environments due to the more complex nature of such environments: substrates are more prone to be adsorbed on particles and therefore less sensitive to the action of microorganisms in soil than in water. Soil is also a non-homogenous medium, its composition varying with depth. Aerobic degradation processes will

* Corresponding author. Tel.: +33 (0)5 62 88 57 31; fax: +33 (0)5 62 88 57 30.
E-mail address: Christine.Cecutti@ensiacet.fr (C. Cecutti).

doi:10.1016/j.biortech.2008.03.050

therefore only be possible within the first few centimeters of the ground's surface. Lastly, although they show greater variety and adaptability, soil microorganisms are different from microorganisms used in aqueous medium tests which mainly originate from the sludge of wastewater treatment plants.

An ISO standard lists different methods available to assess biodegradability in soil (ISO 14239, 1995) however, with the exception of methods implementing the radioactive labeling of the molecules studied, defining a protocol both simple and precise for assessing the biodegradability of chemicals in soil is an uneasy task. Standardisation work is carried out along these lines to define an experimental assessment method of the final biodegradability in soils as easy to implement as the 301 B test. This new method (Cecutti, 2003) based on the principle of the modified sturm test would enable the assessment of the ultimate biodegradability of substances deposited in a bio-reactor containing natural soil with its original microorganisms by measuring the carbon dioxide produced during the biodegradation process. The validation of this experimental test is carried out by correlating the results obtained from both the primary biodegradability values measured in the lysimeters and the ultimate biodegradability values evaluated by OECD 301B method.

Biolubricants vegetable oil based described in literature showed interesting properties toward environment and users (Remmele and Widmann, 1998; Willing, 1999, 2001; Mudge and Pereira, 1999; Gateau et al., 2005), Studies assessing the technical properties of these biolubricants used in various fields have lead to the validation of their use. One of these studies carried out in the field of logging (De Caro et al., 2001) focused on three hydraulic fluids of vegetable origin and one mineral lubricant commonly used in logging machines with the aim to compare the technical characteristics of the two compound families. It seemed interesting to complement this last study by characterising and comparing the environmental qualities of these logging lubricants in terms of both biodegradability (primary and ultimate) and ecotoxicity. These parameters were measured on new hydraulic fluids and interestingly after the logging machines had worked 1000 h in conditions as near as possible to the conditions of their normal use. For the same reasons the doses studied corresponded to the accidental spilling of some hydraulic fluid on the ground, that was approximately 10 l m^{-2}.

2. Methods

2.1. Soils

The soils came from two regions in south-west France. So as to simulate the different conditions in which biolubricants are actually used in forestry, two types of soil with different pedological characteristics were chosen: one sandy soil of acid pH where conifers were cultivated and a second clayey soil of slightly basic pH bearing broad-leaved trees.

2.2. Lubricants

We studied four hydraulic fluids employed in forestry machines: three of them were vegetable based oils classified "environmental friendly lubricants (HE)" according the standard ISO 15380, 2002. They were, colza and/or oleic sunflower esters: a HEES trimethylolpropane ester: Biohydran TMP 46 from FINA firm; and two HETG (triglycérids) hydraulic fluids vegetable oils based: Biolub HETG 346 (colza and oleic sunflower oils based) distributed by IGOL and Hélianthe TRF (oleic sunflower oils based) from TECHNOL firm. The fourth lubricant was a classic mineral based hydraulic, Mobilfluide from Mobil firm, usually employed by the lumberjacks who accepted to participate to the previous demonstration project. This petroleum lubricant, mainly a paraffinic oil, was expected to present interesting environmental properties and allowed the comparison with the three biolubricants.

The assessment of the fluid's ecotoxicity was achieved with new and used fluids, that is fluids having been used 1000 h in a logging machine so as to determine their environmental behaviour in the course of time.

2.3. Lysimeter study: primary biodegradation

We used soils columns also called lysimeter to follow the migration and the progressive primary biodegradation of the lubricants. Lysimeter samples were collected from the two forest sites by coring, which provided undisturbed columns of soil representative of the soil profiles of the sample. At the start of the experiment 300 ml of new lubricants were applied reproducing spillage in forester machines (10 l m^{-2}).

For studies of the kinetics of the lubricants, samples were taken after 30, 60 and 120 days. At each time point, one soil column was analysed for each lubricant. The lysimeters were cut at various depths into slices 5 or 10 cm thick (0–5, 5–10, 10–15, 15–20, 20–25, 25–30, 30–40, 40–50, 50–60 and 60–70 cm). Organic products (lubricants) were extracted from the soil samples by solid/liquid extraction with hexane. The extraction technique was optimal using 200 ml of solvent per 100 g of soil for 6 h. The solvent was evaporated and the lubricants content was determined by gas chromatography with a Varian 3800 chromatograph equipped with an BPX 5 capillary column (Chrompack). The samples were analysed at 22(±2) °C and injected in triplicate.

This method allows to follow their disappearance kinetic by measuring the content in biolubricants in each slice of soil. Biodegradability rates correspond to the accrued values obtained from each section within the 70 cm depth of the lysimeter. We measured also their half-life (DT50), the time required to reduce the amount of a substance by half.

2.4. Ultimate biodegradation tests in aqueous medium and in soil medium

The lubricants' biodegradability evaluation tests were carried out in a liquid medium as in the 301 B test according to the principles of the modified Sturm test A new method based on the principles of the 301 B test was developed in a soil medium this time, making it possible to measure the ultimate stage of the aerobic biodegradation process of the compounds when in contact with the ground: in this new test, the testing reactors contained 1 kg of natural soil with its original microorganisms. The carbon dioxide discharge originating from the ultimate biodegradation of the lubricants introduced in each reactor at a concentration of 200 mg of organic carbon, was measured by titrimetry for the duration of the experiment.

2.5. Ecotoxicity tests

Ecotoxicological effects of lubricants were investigated with standardised test methods commonly required by regulatory authorities for the environmental impact assessment of chemicals:Growth inhibition test with the green algal species *Selenastrum capricornutum* (strain ATCC 2262, algae and protozoa – Cumbria culture collection CCAP no. 27814) were carried out according to OECD Guideline no. 201. Acute toxicity tests with the water flea *Daphnia magna* were carried out according to OECD Guideline no. 202. Acute toxicity tests with the fresh water fish *Brachydanio rerio* were carried out according to OECD Guideline no. 203.

3. Results and discussion

3.1. Primary biodegradability

The degradation rates of the four lubricants in lysimeters after 120 days are listed in Table 1. In both the soils used for this study, biolubricants were better degraded (biodegradation rates between 84% and 88%) than their mineral equivalent (biodegradation rates between 67% and 72%). These results were confirmed by the respective DT 50: 21 to 24 days for biolubricants which led to classify them as slightly persistent, and 35–40 days for the mineral fluid, therefore classifying it as moderately persistent. As an example, Fig. 1 shows the entire lysimeter profile with time of the biolubricant Helianthe in clayey soil. Same tendencies were observed for all lubricants in both soils: an important decrease in the lubricant concentration of approximately 80% was noted in the first thirty days of the experiment. Between 30 and 120 days, the lubricants continued to be degraded and migrated slightly deeper still, concentration peaks moving to the right of the graphs. Whatever the type of soil, the biolubricants of vegetable origin do not migrate as deep as the mineral fluid, but none of the studied compounds migrate further than 60 cm in depth during the 120 days the experiment was carried out. Lubricants were degradated before reach subterranean water. These observations were confirmed by the fact that no traces of lubricants were detected in the water percolating through the sandy soil or the clayey soil during the 120-days experimental period. The risk of water pollution is low even when very large quantities of biolubricants have been spread over the ground as in the event of the rupture of a flexible tube. However, as was expected, the deepest migrations were observed in the sandy soil: approximately 50 cm in depth for the biolubricants and 60 cm for the mineral fluid. The nature of the two soils used in the experiment seems to have no major influence on the degradation and migration of the lubricants, although a slightly better degradation and a less deep migration were observed in the clayey soil than in the sandy soil. These observations may logically be linked and lead to the hypothesis that a slow migration of the product into the ground allows more time for it to be degraded. In the same way, the hydrophobic characteristics of the studied compounds will also be a favourable degradation factor.

Table 1
Primary biodegradation (%) and DT 50 (days) after 120 days in both clayey and sandy soils

Lubricant	Clayey soil		Sandy soil	
	Biodegradation rate	DT 50	Biodegradation rate	DT 50
Biolube	88	21	87	22
Biohydran	87	23	86	22
Helianthe	86	24	84	22
Mobilfluide	72	35	67	40

3.2. Ultimate biodegradability

The ultimate biodegradability analyses showed a decrease in the environmental qualities of these fluids may indeed be feared when fluids may contain wearing metals or may have undergone chemical alterations during their use at high temperatures. Fig. 2 represents by way of example the biodegradation kinetics of used hydraulic fluids in the sandy soil. Similar graphs were obtained with the new fluids in the clayed soil, only the maximum biodegradation rates reached differed. These data were collected in Table 2.

Whatever the type of soil used for the test, a slight decrease in the biodegradation rates for the used lubricants was observed, whether of mineral or vegetable origin and these differences were not significant however as regards their impact on the environment. This behaviour could be explained by the accommodation of the soil microbial communities to the pollutants, despite the chemical alterations of the structures of used lubricants. In all four tests, biolubricants showed very similar degradation kinetics and in all cases more rapid than those of the lubricant of mineral origin. Differences of biodegradability properties between biolubricants and mineral lubricant were more obvious than chemical modifications due to ageing in both kinds of lubricants (Blin et al., 2007).

In the lysimeter tests, slightly different biodegradability rates were observed with sandy and clayey soils, but these differences not occurring however in these biodegradability tests performed in the bioreactors. The laboratory tests were achieved methodically, in two types of very different soils so as to show the impact of lubricants in whatever situation they may be used, as indeed, besides the environmental analysis of the biolubricants, this work was part of a development and validation project of a method of

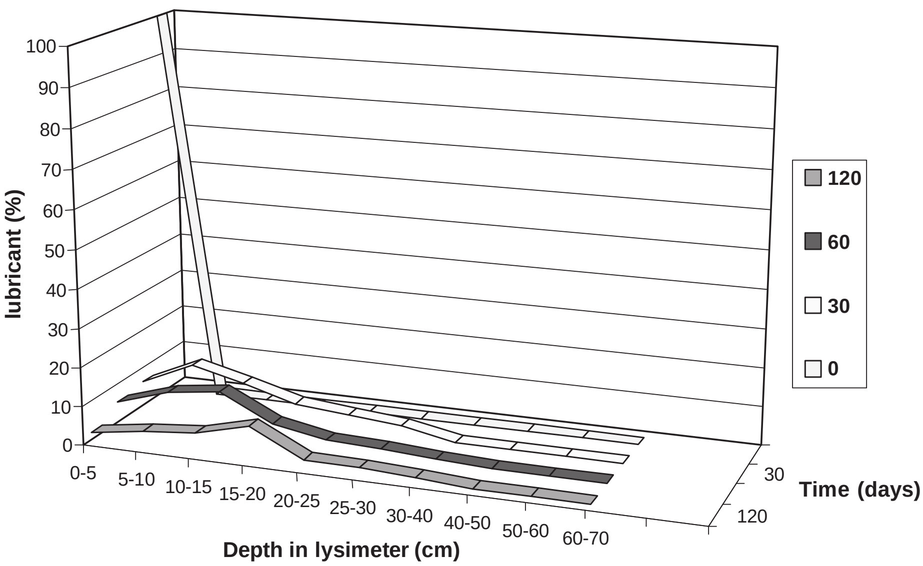

Fig. 1. Changes in the amount of a biolubricant (Helianthe) over space and time in clayey soil.

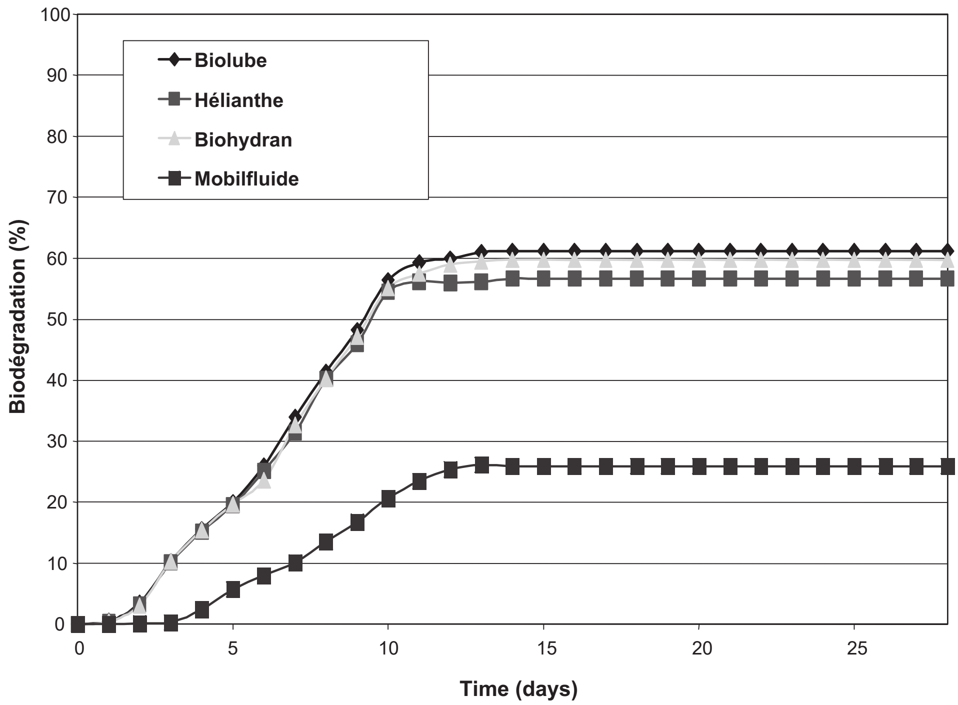

Fig. 2. Ultimate biodegradation of used lubricants in sandy soil.

Table 2
Ultimate biodegradation rate (%) in two soils and in aqueous medium

Lubricant	Biodegradation rate in clayey soil	Biodegradation rate in sandy soil	Biodegradation OECD 301B test
Biolube			
New	64	64	93
Used	62	61	79
Biohydran			
New	63	62	78
Used	61	60	75
Helianthe			
New	61	60	85
Used	57	57	69
Mobilfluide			
New	29	27	93
Used	27	25	78

Table 3
Lubricant ecotoxicity values before and after use

Lubricant	Algae EC50-72 h OECD 201	Daphnia EC50-48 h OECD 202	Fish LC50-48 h OECD 203
Biolube			
New	5400	>10,000	>10,000
Used	5600	5,900	>10,000
Biohydran			
New	2800	>10,000	>10,000
Used	1800	>10,000	>10,000
Helianthe			
New	4800	>9,900	>9900
Used	100	2500	>10,000
Mobilfluide			
New	1300	5400	390
Used	790	2450	380

biodegradability assessment in soil carried out with a normative purpose (Cecutti, 2003).

The aim of this study was therefore to measure biodegradability data that may be reproduced whatever the soil and comparable to the results shown in Table 2, obtained in accordance with the OECD 301B method. Results showed that biodegradation in a liquid medium was more rapid and complete than in a soil environment (Chaineau et al., 1999; Sabourin et al., 1999) and confirm the hypotheses of the better availability of the substrate in a liquid medium than in a soil environment and a better oxygenation of the liquid medium facilitating the aerobic biodegradation process. Nature and origin of inocula have also a great influence on results of biodegradation (Mezzanote et al., 2005) could be an handicap for the use of this kind of test in liquid medium with different activated sludge inocula.

As expected, the mineral oil showed rather good biodegradation rate in liquid medium because of its paraffinic structure. In this case, the biodegradation test in soil medium seems to be more severe than the one in liquid medium.

Furthermore, the difference between new and used fluids is more obvious in a liquid medium due probably to the non accommodation of the microbial population, and the biodegradability rates decrease by approximately 15% or 20% after 1000 h of use. This trend initiated in the soil environment was so largely confirmed in the liquid medium: biodegradability is less complete for used oils than for new ones. This last result may be attributed to the presence of toxic compounds affecting the micro organisms playing a part in the biodegradation process, especially micro organisms from water waste treatment plant. These toxics compounds could be wearing metals, degradation products like acrolein, peroxides or polymers, and in the case of vegetable oil by-products, aldehydic compounds, a consequence of thermal degradation. Synthetic esters studied in this work seem to be preserved from these contamination phenomena due to lubricant wearing as, on the one hand they have a well-known protection role as concerns the wearing of metallic parts and on the other hand, they are more thermally stable than vegetable oils (Willing, 1999) and than mineral oils (Martins et al., 2005).

In all these studies on biodegradability, biolubricants proved to be easily degradable products and showed ultimate biodegradability rates significantly higher than those of the fluid of mineral origin, especially in a soil environment. This superiority is even greater when fluid behaviour is observed after 1000 h of use.

3.3. Ecotoxicity

These tests were achieved with both new and used fluids and results are collected in Table 3. None of the biolubricants were toxic to fishes however their high concentration, that was above 10,000 mg l^{-1} when the fluid of mineral origin was lethal at relatively low concentrations (380 and 390 mg l^{-1}). Tests on daphnia also gave excellent results, of the same order of magnitude for all biolubricants, all the more so when they were new. For Biolube and Helianthe, toxicity increased with the wearing of the fluids but remains acceptable. The mineral fluid was there again the most toxic of all fluids, all the more so after having been used (EC50 = 2450 mg l^{-1}). As in the case of biodegradability, the better behaviour of the synthetic ester biohydran was observed as it will retain its properties having undergone heat and mechanical stresses over a long period (1000 h). The most sensitive test was the test on the algae. It enabled the classification and comparison of biolubricants and underscored an increase in toxicity after use for two of them: Helianthe and Mobilfluide. The toxicity levels of the biolubricants were never high however, even after use, as compared to petroleum-based fluids.

The use of biolubricants in forestry seems to be an excellent way of taking part in the protection of our environment as these easily biodegradable products entails no risk of pollution of soils or subsurface waters. Reference ecotoxical tests also show that they will not be noxious to other surrounding organisms. This work confirms that soil is an excellent medium for biodegradation process because of the great diversity and the facility of adaptation of its microbial populations. Biodegradability tests performed in soil are the best approaches for evaluate of the environmental impact of organic compounds when they are suspected to pollute the soil.

Acknowledgements

We thank ADEME and ONIDOL for their financial support.

References

Agius, D., Cecutti, C., Roques, C., Gaset, A., 1999. Huile adjuvante: devenir des esters méthyliques dans le sol. OCL 6, 396–400.
Battersby, N., 2000. The biodegradability and microbial toxicity testing of lubricants – some recommendations. Chemosphere 41, 1011–1027.
Blin, J., Volle, G., Girard, P., Bridgwater, T., Meier, D., 2007. Biodegradability of biomass pyrolysis oils: comparison to conventional petroleum fuels and alternatives fuels in current use. Fuel 86, 2679–2686.
CEC-L-33A-93, 1995. Biodegradability of two-stroke cycle outboard engines oils in water. Co-ordinating European Council for Development of Performance Tests for Lubricants and Engine Fuels Method.
Cecutti, C., 2003. Un nouveau test d'évaluation de la biodégradabilité dans le sol. Travaux de normalisation. OCL 10 (5–6), 354–359.
Cecutti, C., Agius, D., Caussade, B., Gaset, A., 2002. Fate in the soil of an additive of plant origin. Pest. Manage. Sci. 58, 1236–1242.
Chaineau, C.H., Morel, J., Dupont, J., Bury, E., Oudot, J., 1999. Comparison of the fuel oil biodegradation potential of hydrocarbon assimilating microorganisms isolated from a temperate agricultural soil. Sci. Total Environ. 227, 237–247.
De Caro, P., Gaset, A., Nguyen The, N., 2001. Vegetable based lubricants in forestry: results from a test campaign. Agro-food Industry Hi-Tech, July/August, pp. 26–30.
Eisentraeger, A., Schmidt, M., Murrenhoff, H., Dott, W., Hahn, S., 2002. Biodegradability testing of synthetic ester lubricants – effects of additives and usage. Chemosphere 48, 89–96.
Garcia, M.T., Campos, E., Ribosa, I., 2007. Biodegradability and ecotoxicity of amine oxide based surfactants. Chemosphere 69, 1574–1578.
Gateau, P., Van Dievoet, F., Vermeersch, G., Claude, S., Staat, F., 2005. Environmentally friendly properties of vegetables oil methyl esters. J. Am. Oil Chem. Soc. 12, 308–313.
Haus, F., Germann, J., Junter, G., 2001. Primary biodegradability of mineral base oils in relation to their chemical and physical characteristics. Chemosphere 45, 983–990.
Haus, F., Boissel, O., Junter, G.A., 2003. Multiple regression modelling of mineral base oil biodegradability based on their physical properties and overall chemical composition. Chemosphere 50, 939–948.
ISO 14239, 1995. Soil quality – methods for measuring the mineralization of organic chemicals in soil under aerobic conditions using laboratory incubation systems.
ISO 15380, 2002. Lubricants, industrial oils and related products (class L) – Family H (Hydraulic systems) – specifications for categories HETG, HEPG, HEES and HEPR.
Martins, R., Seabra, J., Brito, A., Seyfert, Ch., Luther, R., Igartua, A., 2005. Friction coefficient in FZG gears lubricated with industrial gear oils: biodegradable ester vs. Mineral oil. Tribiol. Int., 1–10.
Mezzanote, V., Bertani, R., Degli Innocenti, F., Tosin, M., 2005. Influence of inocula on the results of biodegradation tests. Polym. Degrad. Stab. 87, 51–56.
Mudge, S.M., Pereira, G., 1999. Stimulating the biodegradation of crude oil with biodiesel preliminary results. Spill Sci. Technol. Bull. 5, 353–355.
OECD 201, 1984. Guideline for testing chemicals: algal, growth inhibition test. Organisation for Economic Co-operation and Development, Paris.
OECD 301 A-F, 1992. Guidelines for testing of chemicals. Ready biodegradability. Organisation for Economic Co-operation and Development, Paris.
OECD 202, 1994. Guideline for testing chemicals: *Daphnia* sp. acute immobilisation test and reproduction test. Organisation for Economic Co-operation and Development, Paris.
OECD 203, 1994. Guideline for testing chemicals: *Brachidanio rerio*, acute toxicity test. Organisation for Economic Co-operation and Development, Paris.
Pagga, U., 1997. Testing biodegradability with standardized methods. Chemosphere 35, 2953–2972.
Remmele, E., Widmann, B., 1998. Biodegradability and ecotoxicity of hydraulic fluids based on rapeseed oil used in agricultural machinery. Biomass for Energy and Industry, 8–11 June. Wursburg, Germany.
Sabourin, C.L., Carpenter, J.C., Leib, T.K., Spivack, J.L., 1999. Mineralization of dimethylsilanediol by microorganisms isolated of soil. Environ. Toxicol. Chem. 18, 1913–1919.
Willing, A., 2001. Lubricants based on renewable resources – an environmentally compatible alternative to mineral oil products. Chemosphere 43, 89–98.
Willing, A., 1999. Oleochemical esters – environmentally compatible raw material for oils and lubricants from renewable resources. Fett/lipid 6, 192–198.