FINAL TECHNICAL REPORT

PART I: PUBLISHABLE FINAL REPORT

CONTRACT N° : NNE5-2001-00744

PROJECT N° : S07.16365

ACRONYM : BIOTOX

TITLE : An assessment of bio-oil toxicity for safe handling and transportation

PROJECT CO-ORDINATOR : Centre de Coopération Internationale en Recherche Agronomique pour le Développement (Cirad)

PARTNERS : - Aston University
- Bundesforschungsanstalt für Forst- und Holzwirtschaft (BFH)

REPORTING PERIOD : FROM 01 January 2003 TO 30 June 2005

PROJECT START DATE : 01 January 2003 DURATION : 30 Month

Date of issue of this report :

	Project funded by the European Community under the 'Energy FP5' Programme (1998-2002)'

Part I: Publishable Final Report

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0. Executive publishable summary

1 BACKGROUND AND INTRODUCTION

Pyrolysis is one of the three main thermochemical routes to convert biomass into useful primary energy products. Fast pyrolysis has been the subject of active research for approximately the last 25 years in order to obtain optimum yields of bio-oils which can be used in engines for the generation of electricity or after refining in transport. Development has now reached a stage where commercialisation is being attempted. To successfully achieve this, the question of safety procedures for ensuring human health and preservation of the environment needs to be addressed. Within Biotox project bio-oils from all major current producers have been collected to determine factors influencing toxicity, eco-toxicity, mutagenicity and biodegradability.

2 SCREENING TESTS

19 samples were collected from producers utilising fast pyrolysis technologies, namely rotating cone, ablative, fluidised bed and circulating fluidised bed pyrolysis systems. Furthermore, two slow pyrolysis samples were included

2.1 Summary of tests

An overview of the areas that were investigated in the screening tests of the 21 bio-oil samples is shown in Table I. A full description of the test methods will be published in the final project report and will also be available on the PyNe website.

Table I: Details of performed screening tests

Type of study	Detailed analysis
Physico chemical (performed by IWC)	• Chemical composition • Viscosity (at 20 & 50°C) • pH • Density • Stability • Solids content • Water insolubles • PAH • Elemental Analysis C,H,N & O
Toxicological (CIT)	• Bacterial reverse mutation test
Ecotoxicological (CIT)	• Algal growth inhibition test • Acute toxicity in *Daphnia Magna*
Biodegradability (Cirad)	• Modified Sturm test

2.2 Physical and basic chemical properties of bio-oil

All fast pyrolysis oil samples are in the typical ranges for bio-oil physical and chemical properties. Most measured water contents are between 20 and 30%. One sample is significantly higher with a water content of 37%, which may be due to the pyrolysis technology of the sample provider or may just be a sign of high feedstock moisture content. No measurement of the biomass moisture content was supplied with the oil sample. Solids content varies widely, from 0.03% to 3.43%, indicating large variation in char product collection efficiency. The density of all samples is close to 1.2 kg/l.

The viscosity of the samples ranges from 17.5 to 451 cSt at 20°C. Water insolubles, often referred to as pyrolytic lignin, represent between 6% and 25% of the whole oil.

Stability of the oil samples, as assessed by measuring viscosity and water content of the oil again after storage for 24 hours at 80°C, varies greatly, between virtually no change, and a tripling of the viscosity measured at 20°C, or respectively an increase in water content by a factor 1.26.

2.3 Gas chromatography

As can be seen in Fig. 2. chemical species quantified by gas chromatography show substantial variation between the samples. Approximately a quarter of the mass of the wet oil is quantified by the employed gas chromatography methods. The quantified chemicals typically represent 70-80% of the total peak area measured by GC. Several low molecular weight chemicals, for example methanol, acetone and ethanol, are subsumed in the peak of the solvent (acetone) used to dilute the bio-oil in preparation for GC analysis. They are therefore neither quantified nor included in the total peak area, which excludes the very large solvent peak.

2.4 Polyaromatic hydrocarbons

As polyaromatic hydrocarbons are known to represent a potential health and safety concern, a method was developed for analysing their concentrations in bio-oil. It was found that typical concentrations for total PAH are below 10 PPM. PAH concentration is temperature and residence time dependent. The slow pyrolysis oil samples exhibit much higher values for PAH, with one of the two samples slightly exceeding 100 PPM. Bio-oils produced at temperatures exceeding 550°C had the highest PAH values for fast pyrolysis samples, ranging as high as 23 PPM at 600°C. The lowest PAH concentration was found in a sample produced at 425°C.

2.5 Mutagenicity Ames test

For all fast pyrolysis oil samples at least one of the tested strains of Salmonella typhimurium showed a sufficient increase in reverse mutants that the result would be considered a positive indication of mutagenicity according to international regulations. Both slow pyrolysis samples assessed were too toxic for the bacteria for the test to give a result.

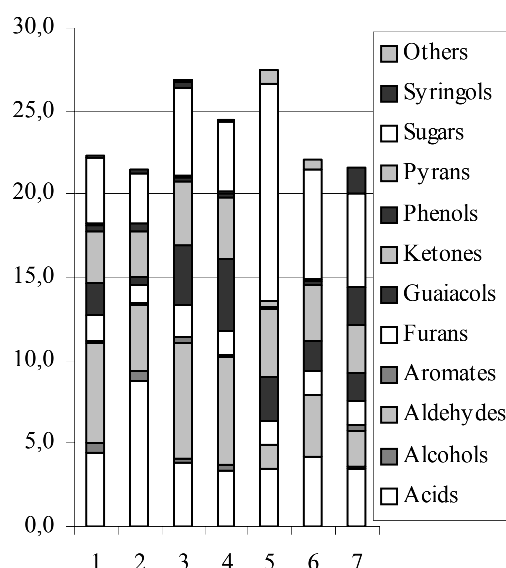

Figure 2: Selection of 7 gas chromatography results, wt% of whole oil, chemical species grouped together

2.6 Ecotoxicological screening

The algal growth test indicates that low concentrations of bio-oil may have a small fertilising effect. The potential to contribute to eutrophication, however, should be small due to the very low nitrogen, and very low to negligible minerals content of the bio-oil. At higher concentrations, algal growth is slightly inhibited.

Acute toxicity on Daphnia Magna, even at the highest loading rate of 100 mg/l, is negligible.

Overall the ecotoxicological screening tests indicate that all tests fast pyrolysis oils are benign in their effects on algae and small water animals.

2.7 Biodegradation

The ready biodegradability of the samples, as assessed through a modified Sturm test, indicates rapid biodegradation of 40-50% of the organic carbon in the oil. These values are better than for most mineral oil products. For example, heavy fuel oil only gives a value of 11% in the modified Sturm test. They are, however, slightly worse than for slow pyrolysis derived oil. This is likely due to the fact that bio-oil contains a large fraction of incompletely decomposed lignin fragments. Lignin is well known to be particularly resistant to microbial decomposition, with only a few species capable of degrading it aerobically, and little degradation being possible anaerobically].

3 COMPLETE SET OF TESTS ON 1 SAMPLE

The preliminary tests indicate little difference between different feedstocks (miscanthus, hardwood, softwood and forest residues with varying bark content) and fast pyrolysis technologies. At higher temperatures PAH is slightly elevated. Consequently, it was decided to do the full suite of tests on a sample produced under well known conditions at Aston University. A temperature of 500°C was chosen, as this is the optimal yield temperature. Furthermore, much lower temperatures, towards 400°C, make it difficult to avoid blockages in the hot piping due to condensation of bio-oil, and temperatures towards 600°C require extra, and more difficult to supply high temperature, process heat.

It was also decided to use a fluidised bed system, as this is currently the most widely used technology, and as repeatability and consistency of results have been particularly well established for this reactor system at Aston.

A softwood was chosen as the feedstock as this is a high quality biomass particularly widely available, at reasonable cost, in the EU.

3.1 Physical and basic chemical properties

In addition to the tests performed for the screening, explosive properties (test A14) were assessed with the conclusion that bio-oil is not explosive. Water solubility (test A6) and partition coefficient (test A8) are difficult to assess for bio-oil. A suitable method is still under discussion with the relevant authorities.

3.2 Acute oral toxicity in rats

At a dosage of 2000 mg/kg no mortality was observed in test B1 tris. Piloerection, the rat's body hair standing on end, which is a potential sign of the rat feeling cold or stressed, was seen within the first 6 hours after treatment. There was also hypoactivity or sedation. No clinical signs were observed on day 2 after treatment.

Test TSR, a seven day study of oral toxicity with the highest treatment at 1500 mg/kg/day, was also performed. Hypersalivation and, at least an initial reduction in food consumption and body weight gain were observed.

These tests indicate no toxicity of bio-oil by oral ingestion.

Table II: Selection of the feasible test to be carried out in the selected bio-oil sample

Study title
PHYSICO-CHIMICAL PROPERTIES
A6 / Water solubility
A8 / Estimation of the partition coefficient
A14 / Explosive properties
TOXICOLOGICAL STUDIES
B1 tris / Oral route - rat
B3 / Cutaneous - rat
B4 / Cutaneous - rabbit
B5 / Eye irritation - rabbit
B6 / LLNA
TSR / 7-day study oral routein rats
MAS / Micronucleus test in vivo
MNV / Micronucleus test in vitro
ECOTOXICOLOGICAL STUDY
C2 / Acute toxicity in daphnia magna

3.3 Dermal irritation in rabbits

In this test (B4) the potential for skin irritation in rabbits is evaluated. Clinical symptoms, such as discoloration, dryness and damage to the skin in the form of lesions or swelling, were observed.

The tested sample therefore has to be classified as corrosive when applied topically to rabbits, and is assigned the symbol C "corrosive" and risk phrase R34 "causes burns". Test B5 (eye irritation in rabbits) was consequently not performed in order to protect the animals. It is also well known that bio-oils are irritating to the eye.

3.4 Evaluation of skin sensitisation potential in mice

Skin sensitisation potential in mice was assessed through test B6 (LLNA or local lymph node assay). This test indicated that bio-oil is a moderate skin sensitiser and therefore has to be assigned risk phrase R43 "May cause sensitisation by skin contact".

3.5 Mutagenicity

Mutagenicity was assessed in two tests (MAS and MNV). In the MAS test the potential of bio-oil to induce damage to the chromosomes or miotic apparatus in bone marrow cells of mice (after 3 oral administrations) is assessed in vivo. In the MNV test mutagenicity is assessed in vitro on mouse lymphoma cells.

Together the tests indicate the potential for slight mutagenicity which would need confirmation through further testing.

4 DISCUSSION

The screening tests indicate a wide variability in chemical composition measured by GC and little difference in toxicological properties of the bio-oils. It is well known that depending on process conditions the relative proportions of sugars, acids and aldehydes vary in bio-oil [8]. These groups contain the chemicals with the highest mass proportions in bio-oil, notably acetic acid, hydroxyacetaldehyde and anhydrosugars, which are known to have low toxicity. The results of the screening tests indicate that the variations in these groups do not materially impact the overall toxicity of the bio-oil, which they could in theory through synergistic or antagonistic effects.

There is an indication that the concentration of PAH may correlate with toxicity, as the two slow pyrolysis oils had both the highest PAH concentrations and showed the greatest toxicity. PAH are also well known to contribute to health and safety concerns in conventional petroleum derived fuels.

The results also provide relevant information for the labelling, storage and transportation of bio-oils. They indicate that the oils do not need special precautions in terms of explosive concerns, or toxic or ecotoxic emissions. They are, however, corrosive and irritating to skin and therefore require appropriate personal protective equipment during handling.

The data generated in this work can also be used to help produce an MSDS sheet and technical dossier for bio-oil as required by incoming European legislation on chemicals control, as will be discussed in detail in the final project report.

5 CONCLUSIONS

The results indicate that in spite of substantial variations in the proportions of some chemicals contained in different bio-oils, all fast pyrolysis processes give bio-oil that is very similar in terms of toxicity, eco-toxicity and biodegradability.

Bio-oil appears to be more benign than slow pyrolysis derived tars, although it takes longer to biodegrade. In comparison to traditional petroleum derived fuels bio-oil biodegrades faster, and is considerably less toxic.

I. Publishable synthesis report

I.1. Objectives and strategic aspects

I.1.1 Social and economic objectives of the project.

Pyrolysis is one of the three main thermochemical routes to convert biomass into useful primary energy products. It consists in the heating of a raw material in absence of oxygen. As a result of the thermal decomposition of the raw material, a gas, a liquid and a solid are formed, which can be used directly or further upgraded to give more value-added fuels. Fast pyrolysis at a temperature around 500°C, at very high heating rates and short vapour residence times (less than 1 second) gives high liquid yields of up to 80% weight on a dry feed basis. Fast pyrolysis is therefore unique in that a liquid product is produced in high yields in a simple one step process with all the advantages offered by a liquid fuel. The White Paper edited by the Commission has described biomass as the most important source of renewable energy for the future. In the long term, biomass will undoubtedly play a significant role in the supply of energy in many countries. The project intends to assess and minimize the effect of a liquid fuel (bio-oils) from fast pyrolysis of biomass, on human health and its impacts on environment, before large scale marketing and utilisation, by acting on the production parameters. It aims at producing cleaner bio-oils to both at local and global scale.

The development of the use of bio-oils as fuels will have a positive impact on the reduction of CO_2 emissions as they derive from biomass (CO_2 neutral).

More generally speaking, the development of the use of bio-oils will increase the use of European forest and agricultural products and by products. From an economic viewpoint, this will induce job creations in rural areas, thus developing economic and social welfare. The most promising application of bio-oils is electricity production, or combined heat and power, due to their ability to be used in an engine without extensive upgrading as well as the ability to decouple the fuel production source from the end-use location. Small size (a few MWe) decentralised electricity production can be achieved, which will allow the development of activities in less favoured regions.

An other very attractive option offered by fast pyrolysis is transport fuel application through bio oil gasification and Fischer Tropsch reaction as fast pyrolysis offers a unique advantage of decoupling the production phase of its use as well as generating standard fuel form dispersed and divers biomasses.

Adapted health and safety procedures are important and will have running cost reduction implication as it will allow appropriate risk evaluation and a better insurance coverage. More over a better understanding of operating conditions responsible for more severe toxicity and eco-toxicity will improve day-to-day operation and reduce losses.

I.1.2 Scientific/technological objectives of the project.

Fast pyrolysis has benefited from active research programme since 1980's because bio-oils can be substituted directly for fuel oil in many static applications or used as a source of renewable chemicals and in the longer term it can be upgraded for more demanding applications such as transport fuel. Today, different demonstration plants are set up in Europe as well as in North America and significant quantities of bio-oils are produced for research and development purposes. Thus, the question of safety procedures for human health and environment preservation is raised during production, transport and use of the bio-oils. Indeed bio-oils contain 100s of chemicals from different functional groups, such as organic acids, aldehydes and ketones, phenolic compounds, aromatics. Their composition mainly depends on the feedstock used and the pyrolysis conditions. Thus, the toxicology of the oils also

depend on feedstock and process. No systematic studies were made to relate the different parameters. This was mainly due to the high cost of the necessary toxicological and biodegradability studies, which prevents their financing by generally SMEs fast pyrolysis companies.

Moreover with the increasing amount of bio-oils manufactured, transported (imported) and stored within the EU market, it was necessary to register (notify) this substance by competent authorities with a comprehensive and definitive MSDS and proper preventative and remedial procedures to adopt during production, transport and use of bio-oils.

Base on these observations during the project, the relation between process parameters on one hand and chemical composition and toxicity for human health and environment on the other hand were investigated. Then, a bio-oil, selected to be representative of those placed in the Eu market, was submitted to mandatory tests required by the commission, the objective being the definition of secure handling and storage procedures, in order to control the risks related to the product for the population and the environment. The effects of different ways of exposure (inhalation, ingestion or skin contact) were quantified, as well as the effects of long term exposures. The impacts on the environment was also be evaluated by biodegradability, and effects on bio-organisms.

The aims of this project were:

- to determine toxicological and eco-toxicological data on bio-oils. The work will concern both acute and chronic effects on human health, carcinogenic and mutagenic effects. The environmental impacts of bio-oils liquids will also be measured in water and soil. This will allow a comprehensive and definitive MSDS to be produced with the proper preventative and remedial procedures to adopt during production, transport and use of bio-oils,
- to determine the best operating conditions to avoid or minimise the formation of toxic products from the composition of the bio-oils,
- to produce fast pyrolysis bio-oils with low impact on human health and environment by optimising the production process of bio-oils related to toxicity characteristics,
- to encourage the active involvement of industry in the development and exploitation of the results through an existing network, which includes developers of bio-oils production and application technologies.
- to disseminate information on this activity from the database and results of meetings by newsletters, reports, conferences, specific workshops, web site and other publications, to those of the network and other interested parties in regional, national and international programmes.

I.2. Scientific and technical description of the results

Fast pyrolysis is a high temperature process in which biomass is rapidly heated in the absence of oxygen. As a result it decomposes to generate mostly vapours and aerosols and some charcoal. Liquid production requires very low vapour residence time to minimise secondary reactions of typical 1s, although acceptable yields can be obtained at residence times of up to 5s if the vapour temperature is kept below 400°C. After cooling and condensation, a dark brown mobile liquid is formed which has a heating value about half that of conventional fuel oil. Moreover, biomass is a complex mixture of hemicellulose, cellulose, lignin and minor amounts of other organics which pyrolyses or degrades at different temperatures and by different mechanisms and pathways. The rate and extent of decomposition of each of these components depends on the process parameters of reactor (pyrolysis) temperature, biomass heating rate and pressure. The degree of secondary reaction (and hence the product yields and characteristics) of the gas/vapour products depends on the time-temperature history to which they are subjected before collection, which includes the influence of the reactor configuration.

Pyrolysis, has received considerable creativity and innovation in design reactor systems that provide the essential ingredients of high heating rates, moderate temperatures and short vapour product residence times for liquids. As a result a large variety of reactor configurations has been developed and is used by bio-oil producers.

Project partners decided to study the impact of the pyrolysis parameters on the nature of the obtained oils trough assessment of the physicochemical composition and the toxicological impact of oils samples produced under different conditions and in diverse reactors. Goal of this work was to give a overview of the characteristics of the oils produced within the Eu market, and based on the whole results to select one representative oil to be fully analysed in term of toxicity and ecotoxicity.

Methodology applied during the project is presented on schema Figure 1. At the beginning of the project, 21 selected samples of bio-oils from different processes, temperatures and feedstock were gathered, in order to be analysed in screening through physicochemical and toxicological tests. Results of these screening tests were collected to correlate bio-oils production parameters to compositions and toxicological characteristics, and to select the most representative sample to be used for full toxicological analysis through mandatory tests required by the EU legal authority; the objective being the definition of secure handling and storage procedures, in order to control the risks related to the product for the population and the environment.

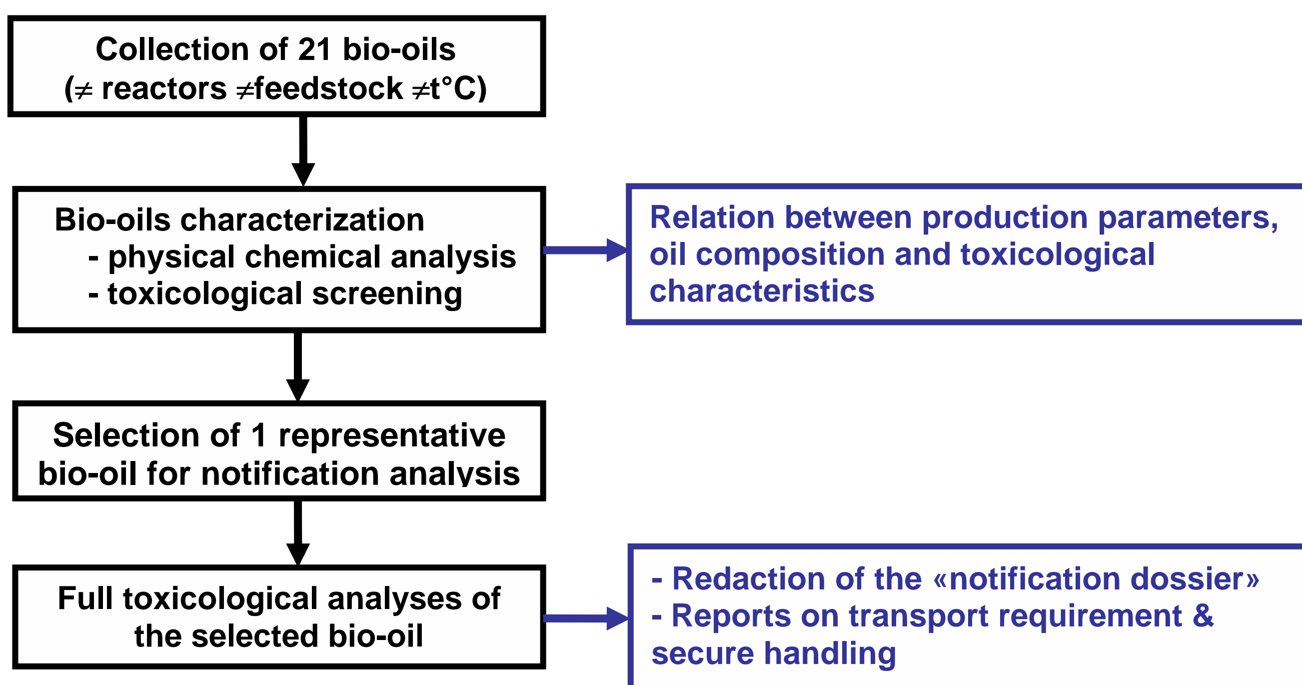

Figure 1: Schematic methodology applied for the project

The whole work carried out within Biotox was shared in four successive phases:

1. Selection, production and collection of 21 bio-oil samples to be assessed
2. Assessment of the physico-chemical and the toxicology properties of the collected samples
3. Full toxicological study of one selected representative sample
4. Recommendations for safety procedures and dissemination of the results

I.2.1 Selection, production and collection of 21 bio-oil samples to be assessed

As bio-oils producers are the main beneficiary of the results of the project, they were implied into the project at the beginning. In order to involve them to the project, they were invited to the first Biotox steering committee meeting held in Paris in February 2003. The project was also introduced to the European pyrolysis network “PyNe” during the Florence meeting in April 2003. Through these meetings industrialists were informed about the objectives of the project and the methodology to reach them. Industrialists confirmed their interests and their willingness to take part to the project. All of them were favourable to provide bio-oil samples.

A first series of fourteen samples was collected for the screening tests and based on the first analytical results; it has been decided to produce 7 additional samples in order to assess the reproducibility of the tests, to further investigate the effect of temperature and assess aging of sample. A total of 21 samples provided by different laboratories or companies were collected for physicochemical analysis and toxicological assessment.

As shown on Table 1, different types of pyrolysis processes and biomass feedstock were selected in order to provide representative samples for the project and to meet, as well as possible, the industrialists’ needs.

Sample nb°	T°	Parameters	Biomasse
Biotox - 1	**520°C**	Fluidised Bed	Spruce
Biotox - 2	**500°C**	Circulating Fluidised Bed	Beech
Biotox - 3	**500°C**	Circulating Fluidised Bed	Green forest residue
Biotox - 4	**480°C**	Circulating Fluidised Bed	Pine sawdust
Biotox - 5	**460°C**	Fluidised Bed	Pine/Spruce/Fir
Biotox - 6	**560°C**	Ablative	Pine
Biotox - 7	**475°C**	Slow pyrolysis	Residues of soft wood barks
Biotox - 8	**500°C**	Slow pyrolysis	Spruce chips
Biotox - 9	**500°C**	Fluidised Bed	Beech
Biotox - 10	**500°C**	Fluidised Bed	Spruce
Biotox - 11	**425°C**	Fluidised Bed	Spruce
Biotox - 12	**600°C**	Fluidised Bed	Spruce
Biotox - 13	**500°C**	Fluidised Bed	Miscanthus
Biotox - 14	**600°C**	Ablative	Spruce
Biotox - 15	**575°C**	Fluidised Bed	Spruce
Biotox - 16	**425°C**	Fluidised Bed	Spruce
Biotox - 17	**500°C**	Fluidised Bed	Beech
Biotox - 18	**500°C**	Ablative	Beech
Biotox - 19	**500°C**	Fluidised Bed	Spruce
Biotox - 20	**600°C**	Biotox 17 +Biotox 18 +Biotox 19	Spruce
Biotox - 21	**500°C**	Fluidised Bed	Spruce

Table 1: List of collected pyrolysis oils sample

In the selection of samples for the screening tests, the main methods of achieving pyrolysis were represented (Fluid bed, Circulating Fluid Bed, Ablative and slow pyrolysis)

In order to assess the impact of the nature of the feedstock and the pyrolysis temperature on the composition and toxicology of bio-oils these parameters were studied:

- Samples Biotox-10;-11;-12;-15;-16;-21, were produced under the same condition just varying the pyrolysis temperature from 425°C to 6000°C.
- Samples Biotox-9;-10;-13;-17;-21, were prepared from different biomass; beech (hard wood), spruce (soft wood) and miscanthus (perennial grass).

To assess the reproducibility of pyrolysis processes and of the physico-chemical analysis two bio-oil samples (Biotox-9 and -10) were produced in double (respectively Biotox-17 and -21).

As bio-oils are achieved through thermo-chemical degradation of the macromolecules in biomass (hemicelluloses, cellulose and lignin) some obtained compounds in the oils can react during storage with the time to give new longer components. To study this effect sample Biotox-10 was analysed twice, at 4 and 16 months after its production respectively (Biotox-10 and 16).

All samples were collected by Cirad before being distributed between BFH, CIT and Cirad for analysis.
For each sample, oil producers had to fill out a sample data sheet, with all the information concerning the pyrolysis run. This data sheet was collected by Cirad together with the oil sample.
Seven of the twenty-one oils were produced by industrialists in their own equipments.

I.2.2 Assessment of the physico-chemical composition and the toxicology of the collected samples

Physico-chemical composition, toxicity and eco-toxicity of the collected samples were assessed by basic tests before carrying out a comprehensive full toxicological study on one sample
Main goals of this work were to:

- obtained complete description of the chemical and physical properties of the 21 bio-oils
- evaluate effects of bi-oils on bio-organisms (fauna and flora) and their persistence in the environment
- relate for each oil the production parameters to toxicology.
- to select a representative sample for comprehensive full toxicological studies, based on the results of the tests carrying out on the 21 bio-oils,

All the collected bio-oils were first distributed between BFH, CIT and Cirad for analysis.
The Institute of wood chemistry (BFH) analysed the chemical and physical composition of bio-oils.

Toxicological and eco-toxicological test were performed by CIT. This laboratory is an independent laboratory which is legally recognizes to carry out environment, health and safety mandatory tests in Europe. In order to select relevant test for the screening of bio-oils, a meeting was organized in May 2003 in CIT headquarter in Evreux (see minute meeting n°2). Based on the bibliography and objectives of Biotox, Mrs De Jouffrey from CIT, discussed the different tests which would offer the maximum of information during the screening test and suggest to put emphasis on :

- Toxicological test : mutagenicity ames test, as controversial data appears in bibliography
- Eco-toxicological tests: acute screening toxicity study in daphnia and algae due to the environmental benefit expected from the Oil.

Cirad carried out biodegradability tests**.** Due to the specificities of Bio-oils (low solubility in water, high carbon content and viscosity) a new method was developed to asses their aerobic degradation in fresh water.

Details of all analytical tests performed on the 21 bio-oils is summarised in
Table 2.

Type of study	Detailed analysis	Laboratory
Physico chemical	• Chemical composition	**BFH**
	• Viscosity (at 20 & 50°C)	**BFH**
	• pH	**BFH**
	• Density	**BFH**
	• Stability	**BFH**
	• Solids content	**BFH**
	• Water insoluble content	**BFH**
	• PAH	**BFH**
	• Elemental Analysis C,H,N & O	**BFH**
Toxicological	• Bacterial reverse mutation test	**CIT**
Eco-toxicological	• Algal growth inhibition test	**CIT**
	• Acute toxicity in *Daphnia Magna*	**CIT**
Biodegradability study	• Modified Sturm test	**Cirad**

Table 2: Details of tests performed

I.2.2.1 Bio-oils physico-chemical characterisation

BFH was responsible of the physico-chemical analysis Bio-oils. The objectives of this work were:

- to obtained complete description of the bio-oils with chemical and physical properties
- and to determine the range of variation of bio-oils characteristics upon feedstock, temperature and reactor technology

In order to improve and verify the analytical methods used for the physico-chemical characterisation of bio-oils, BFH worked in the framework of the project on the development of new techniques. In order to verify the analysis methods, a same bio-oil sample was analysed, at the beginning of the project (summer 2003) by BFH and Cirad.

The main tool for the analysis of the bio-oils is gas chromatography (GC).The GC method applied in this project was developed at BFH and has been used also for the analysis of liquid smoke aromas. It is recommended by the EU Joint Research Centre (Institute for Reference Methods and Materials, IRMM) for liquid smoke producers and other related research laboratories. A method to analyse PAH was newly developed and agreed with the European Joint Research Centre. The most problematic step was the extraction of the PAH's from the matrix bio-oil. A dedicated GC/MS system with single ion monitoring (SIM) was necessary to obtained the necessary low detection levels.

a) Chemical and physical characterization

In the course of project 21 bio-oil samples were fully characterized by the following parameters:

1. Water (%)
2. Viscosity 20°C (cSt)
3. Viscosity 50°C (cSt)
4. Density 20 °C (g/cm^3)
5. Solids (%)
6. water insolubles (pyrolytic lignin)
7. Stability
8. Viscosity index 20 °C
9. Viscosity index 50 °C
10. Water index
11. Elemental analysis
12. GC analysis
13. PAH determination
14. UV/Vis spectra

- Physical-chemical analysis

The results of the physical-chemical analyses are summarized in Table 3.

	Biotox-1	**Biotox-2**	**Biotox-3**	**Biotox-4**	**Biotox-5**	**Biotox-6**	**Biotox-7**	**Biotox-8**	**Biotox-9**	**Biotox-10**	**Biotox-11**	**Biotox-12**	**Biotox-13**	**Biotox-14**	**Biotox-15**	**Biotox-16**	**Biotox-17**	**Biotox-18**	**Biotox-19**	**Biotox-20**	**Biotox-21**
Water (%)	23.50	28.50	29.10	24.90	29.40	37.00	8.10	28.60	26.80	22.40	26.70	20.30	24.60	22.70	12.12	18.23	25.51	31.79	22.95	26.77	17.57
Viscosity 20°C (cSt)	128.90	118.30	49.30	62.40	47.60	17.50	n/a	n/a	29.10	90.30	81.00	451.30	110.20	112.30	n/a	359.10	41.57	52.78	191.25	39.36	226.43
Viscosity 50°C (cSt)	11.60	3.30	9.90	11.00	10.50	4.90	n/a	n/a	6.90	14.30	14.60	41.70	14.80	16.40	n/a	39.36	8.46	4.70	23.10	8.45	27.96
Density 20°C (g/cm^3)	1.21	1.20	1.20	1.21	1.22	1.19	1.21	1.09	1.16	1.20	1.22	1.21	1.17	1.21	n/a	1.27	1.16	1.14	1.21	1.17	1.25
Solids (%)	0.03	3.43	0.37	0.10	0.03	0.35	2.48	0.03	0.06	0.03	0.52	0.55	0.48	0.40	0.24	0.04	0.74	0.08	0.02	0.09	0.10
Water insoluble	19.7	17.7	12.4	15.2	10.9	5.8	54.4	?	18.2	19.1	15.6	24.9	20.2	21.1	n/a	14.9	16.2	11.3	20.7	15.2	15.7
Stability																					
visc. 20°C (cSt)	256.9	n/a	31.1	97.1	44.7	n/a	n/a	n/a	43.6	262.7	154.0	n/a	190.9	142.0	n/a	621.3	n/a	20.8	332.2	80.1	173.7
visc. 50°C (cSt)	27.4	n/a	10.6	16.8	10.4	n/a	n/a	n/a	9.0	28.8	19.1	n/a	23.5	36.3	n/a	58.8	n/a	0.6	32.9	12.5	52.2
water (%)	24.4	35.3	31.5	26.5	30.6	41.4	n/a	n/a	27.6	24.3	29.2	20.8	25.6	28.7	n/a	19.2	28.4	33.7	24.9	28.8	19.1
Viscosity index 20°C	0.99	n/a	-0.37	0.56	-0.06	n/a	n/a	n/a	0.50	1.91	0.90	n/a	0.73	0.26	n/a	0.73	n/a	-0.61	0.74	1.04	-0.23
Viscosity index 50°C	1.36	n/a	0.07	0.53	-0.01	n/a	n/a	n/a	0.30	1.01	0.31	n/a	0.59	1.21	n/a	0.49	n/a	-0.87	0.43	0.47	0.87
Water index	0.04	0.24	0.08	0.06	0.04	0.12			0.03	0.08	0.09	0.02	0.04	0.26	n/a	0.05	0.11	0.06	0.08	0.08	0.09
CHO, total oil as received																					
% N	0.00	0.01	0.30	0.01	0.35	0.35	0.25	0.00	0.00	0.00	0.37	0.38	0.22	0.42	0.50	0.04	0.38	0.21	0.05	0.61	0.36
% C	43.24	39.45	39.44	41.27	38.28	32.64	59.38	62.54	41.67	43.66	39.37	45.10	43.13	45.18	47.43	44.05	42.59	37.38	43.14	40.78	44.59
% H	7.76	7.96	8.01	7.79	7.77	8.30	7.46	7.59	7.87	7.67	7.64	7.35	8.14	7.60	6.87	7.10	8.09	8.48	7.64	8.07	7.12
% O	49.00	52.58	52.25	50.93	53.60	58.71	32.91	29.87	50.46	48.67	52.62	47.17	48.51	46.80	45.20	48.81	48.94	53.93	49.17	50.54	47.93
	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
CHO, organic part of oil																					
% N	0.00	0.01	0.42	0.01	0.50	0.56	0.27	0.00	0.00	0.00	0.50	0.48	0.29	0.54	0.57	0.05	0.51	0.31	0.06	0.83	0.44
% C	56.52	55.17	55.63	54.95	54.22	51.81	64.61	87.59	56.93	56.26	53.71	56.59	57.20	58.45	53.97	53.87	57.18	54.80	55.99	55.69	54.09
% H	6.73	6.70	6.74	6.69	6.38	6.65	7.14	6.18	6.68	6.68	6.38	6.39	7.17	6.57	6.29	6.21	7.06	7.25	6.61	6.96	6.27
% O	36.75	38.11	37.21	38.34	38.90	40.99	27.98	6.23	36.39	37.06	39.41	36.54	35.34	34.44	39.17	39.87	35.26	37.64	37.34	36.52	39.20
	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

n/a not applicable due to sample characteristic

Table 3: Summary of physical-chemical analyses of all Biotox oils

Most of the oils show typical values for fast pyrolysis liquids.

The variation in water content is visualized in Figure 2. Water content ranges between 8 and 37 %. The highest water content shows bio-oil 6 and the lowest can be found in Biotox-7.

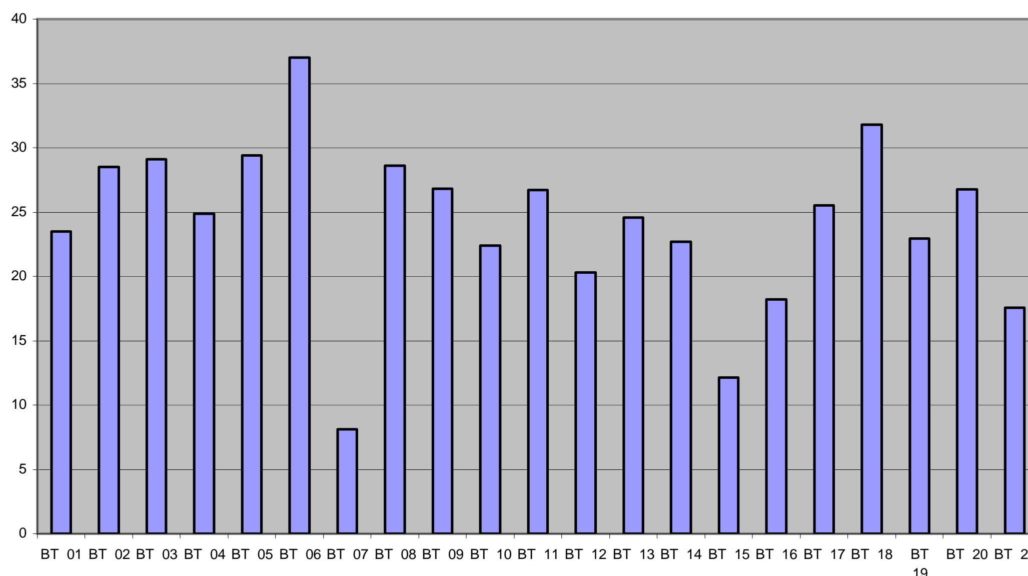

Figure 2: Water content (wt.%) of bio-oil samples

Biotox-6 is from an ablative process which used wood with a moisture content of 14 %. Together with possible slight cracking reactions on the hot surface of the reactor this might explain the high water content. The low water content of Biotox-7 is probably due to the two stage condensation process where the water is condensed in the first stage and most of the organics in the second stage. Biotox-18 shows also water content above 30 %. This oil is made in a rotating cone reactor. It is important to note, that the water content cannot be used as indicator to assess a pyrolysis reactor. The water content in the oil is much more dependent on the condensation system and operating conditions.

Figure 3 demonstrates the viscosity of the bio-oils. Two oils (Biotox-7 and Biotox-8) could not be measured with the capillary viscosimeter because of handling difficulties caused by very high viscosities at room and elevated temperatures. Most of the oils are in the normal range (50-120 cSt) but oil Biotox-12 exhibits extraordinarily high values of 451 cSt without clear explication.

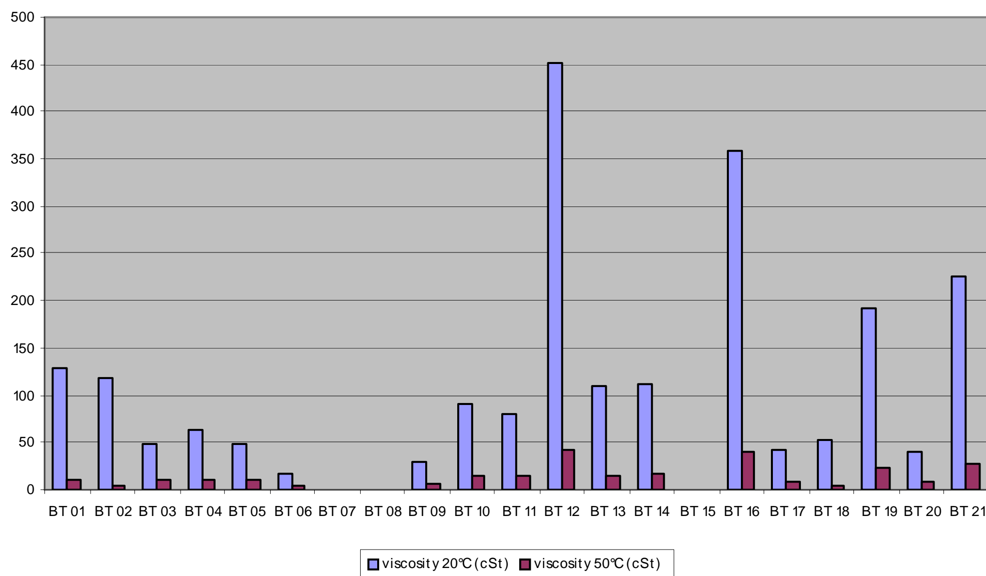

Figure 3: Viscosity at 20 and 50 °C

Density is for all bio-oils in the normal range of 1.2 gcm^{-1} while the solid content varies a lot between 0.03 to 2.5 %. Solid content is mainly function of the condensation device of the pyrolysis unit.
The samples Biotox-2 and Biotox-7 have very high solids contents, 3.43 and 2.48, respectively. Biotox-2 was made in a circulating fluidized bed reactor and some sand might be entrained into the condensation system for bio-oil. For Biotox-7 the high solids content can be explained by the choice of feedstock (pine bark) which is rich in condensed phenolics which might not be completely solubilized in ethanol which is the normal solvent for bio-oils for the determination of the solid content.
The water insoluble part resembles the pyrolytic lignin fraction which describes the oligomeric part of the oil derived from lignin. It ranges from 5.8 to 54.4 %. Biotox- 6 from ablative pyrolysis has only 5.8 % lignin which could be explained by cracking reactions. This finding explains the high water content in this oil. On the other hand, Biotox-7 contains 54.4 % which can easily be explained by the used feedstock (bark) and the two stage condensation system in which lighter molecules are separated in the first stage.

The water index is a measure of the water increase after the stability test, which comprises the treatment of bio-oils for 24 h at 80 °C. The more water is formed, the higher is the index number. From
Figure **4** it can be seen that oil Biotox-14 has the highest water index number, followed by Biotox-2, Biotox-16, Biotox-17, and Biotox-11.

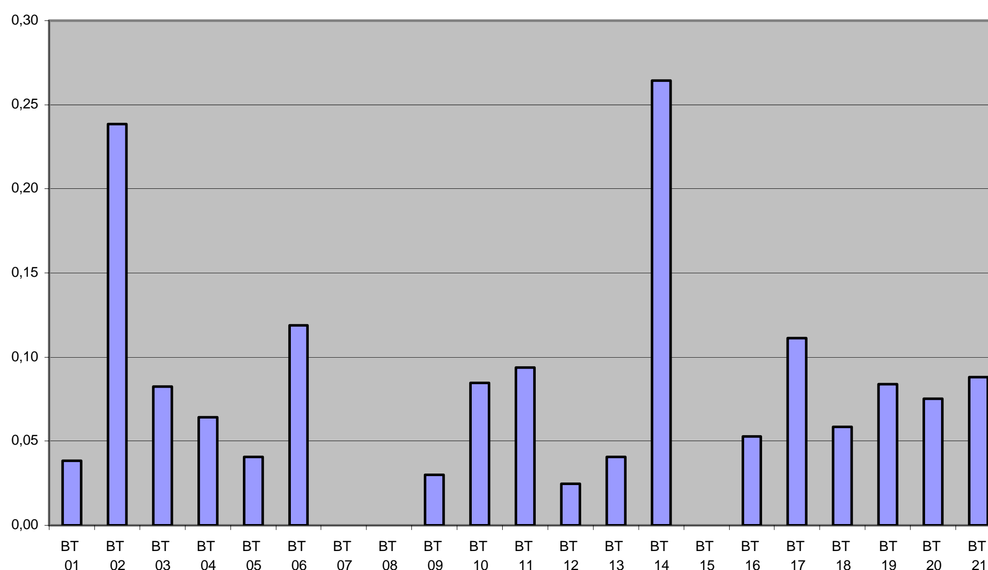

Figure 4: Water index of bio-oils

- Gas chromatographic analysis

GC analysis was performed using standard conditions developed at BFH. Separation takes place on a medium polar capillary column. The overall results are presented in Table 4. The identified single components were clustered into chemically different groups.

The GC method used in this project will be mandatory for future liquid smoke analysis required by the European Joint Research Centre (JRC), Institute for Reference Materials and Methods, Geel, Belgium.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
Identified peaks %	65.7	68.5	74.0	70.3	75.7	74.2	61.5	70.6	75.4	72.8	78.4	71.7	70.4	72.3	84.9	80.1	93.4	89.3	92.5	92.7	93.9
Unknown peaks %	34.3	31.5	26.0	29.7	24.3	25.8	38.5	29.4	24.6	27.2	21.6	28.3	29.6	27.7	15.1	19.9	6.6	10.7	7.5	7.3	6.1
Groups (wt. %)*																					
Acids	4.4	8.8	3.8	3.4	3.4	4.2	3.5	4.1	7.2	2.8	3.2	3.0	4.9	5.7	2.3	2.3	6.0	5.1	2.5	5.2	2.2
Alcohols	0.5	0.5	0.2	0.3	0.0	0.0	0.1	0.0	0.6	0.2	0.1	0.3	0.1	0.4	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Aldehydes	6.0	4.0	7.0	6.5	1.4	3.8	2.1	0.9	3.9	7.0	5.1	6.1	5.9	5.3	17.8	7.4	3.7	2.4	3.5	3.6	6.8
Aromates	0.1	0.1	0.3	0.0	0.0	0.0	0.4	0.3	0.3	0.3	0.0	0.0	0.0	0.3	0.0	0.0	0.1	0.0	0.0	0.0	0.0
Furans	1.6	1.1	1.9	1.5	1.5	1.4	1.4	2.3	2.0	2.2	3.0	2.4	2.0	1.4	1.3	1.2	1.2	0.9	1.0	1.0	1.4
Guaiacols	1.9	0.5	3.6	4.3	2.6	1.9	1.7	8.1	1.8	4.2	3.9	3.2	1.8	2.8	2.6	2.3	1.0	1.5	2.2	1.6	2.6
Ketones	3.1	2.7	3.8	3.8	4.0	3.4	2.9	5.5	4.1	3.5	5.8	4.3	4.5	4.6	3.3	3.0	3.2	2.7	2.4	3.0	2.9
Phenols	0.4	0.4	0.3	0.2	0.2	0.3	2.2	2.3	0.3	0.4	0.2	0.6	1.1	0.5	0.4	0.1	0.2	0.2	0.2	0.2	0.2
Pyrans	0.0	0.0	0.1	0.1	0.3	0.0	0.0	0.0	0.1	0.1	0.4	0.5	0.7	0.3	0.3	0.1	0.2	0.1	0.0	0.1	0.1
Sugars	4.0	3.0	5.2	4.2	13.1	6.6	5.7	1.1	3.4	4.6	5.7	5.9	5.3	3.6	2.6	3.0	2.8	2.8	2.9	2.8	2.5
Syringols	0.0	0.3	0.4	0.1	0.0	0.0	1.6	0.0	3.3	0.2	0.0	0.0	1.6	2.2	0.0	0.0	3.3	3.1	0.0	2.5	0.0
Others	0.1	0.0	0.1	0.1	0.9	0.6	0.0	0.5	0.0	0.1	0.6	1.4	0.8	0.6	0.1	0.2	0.1	0.1	0.1	0.1	0.2
Total	**22.3**	**21.5**	**26.9**	**24.5**	**27.5**	**22.0**	**21.6**	**25.1**	**26.9**	**25.7**	**27.8**	**28.1**	**28.7**	**27.8**	**30.6**	**19.6**	**21.8**	**18.8**	**14.9**	**20.2**	**18.8**

* wt.% based on wet oil as received

Table 4: Summary results of GC analysis

The gas chromatographic results show that maximum 30.6 wt% of the whole could be identified by GC corresponding to 70-95 % of the total GC-peak area.

The amounts of identified and unidentified peak areas are presented in Figure 5.

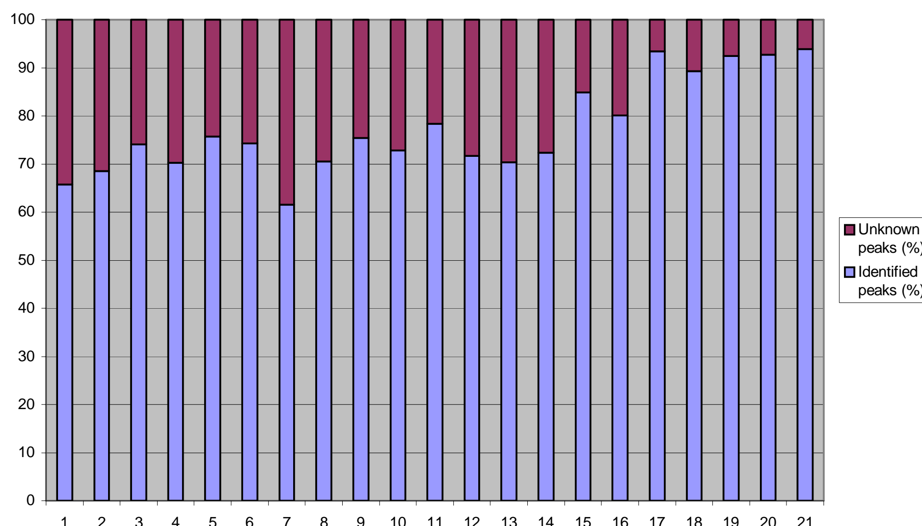

Figure 5: Percentage of identified and unidentified peaks based on the total gas chromatographic area

Oils Biotox-17 to Biotox-21 exhibit the highest identified amount (92-95 %), whereas oil Biotox-1 and Biotox-7 have the lowest identified portion of 62-65%.

Figure 6 shows the distribution of chemical groups in the data set of 21 bio-oils. Predominant groups are acids, aldehydes, phenols, and sugars. Variation in of these groups within the oils is quite substantial and reflects the different processing parameters such as pyrolysis technique and condensation mode.

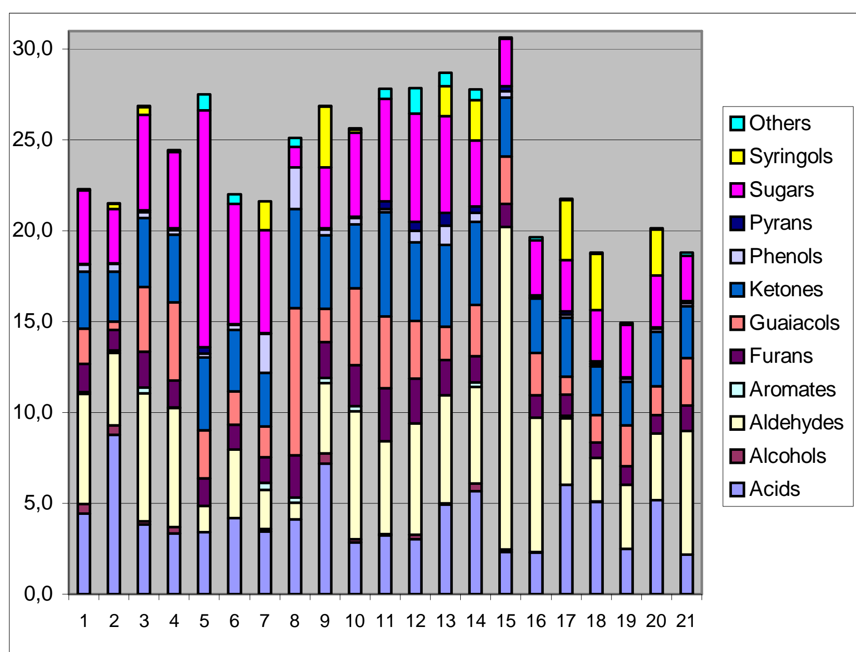

Figure 6: Distribution of chemical groups in the bio-oils (wt.% based on wet oil)

Samples Biotox-16 to -21 give 5-10 % less yield mainly due to 2 reasons:

1. At BFH the sample amount for analysis was adopted by taking into account the different water contents. For samples 1-15 always 60 mg of sample were diluted with 1 ml of acetone regardless the different water contents. This procedure led to the problem, that peak areas were often too high or too small and fell out of the calibration range. Therefore, sample 15-21 were weight in such a way, that always the same organic concentration was used for sample dilution, taking into account the water content.

   An error in calibration could be excluded as all data were crosschecked. As an example the area of the internal standard and hydroxyacetaldehyd is demonstrated in
   Table **5** showing only small variation in the area of the internal standard peak.

Biotox	RT	Area Hydroxyacetaldehyd	Area Internal Standard
1r1	6.255	682323	305455
1r2	6.264	595758	305985
2r1	6.246	436699	301644
2r2	6.21	460930	312260
3r1	6.066	673015	292142
3r2	6.066	630364	280806
3r3	6.066	649367	283249
4r1	6.264	635401	292520
4r2	6.255	644617	295873
5r1	6.921	119532	295243
5r2	6.885	165268	283354
6r1	6.894	278112	252300
6r2	6.894	308401	253305
7r1	6.039	218146	288248
7r2	6.048	206653	291135
7r3	6.048	207618	285047
8r1	6.066	79120	281034
8r2	6.057	75029	281461
8r3	6.039	75554	287073
9r1	6.246	359548	308607
9r2	6.228	347723	310951
10r1	6.066	620199	289728
10r2	6.066	675975	281066
10r3	6.066	654678	285446
11r1	6.822	377959	253663
11r2	6.813	393844	268642
12r1	6.822	505283	273225
12r2	6.831	502952	274304
13r1	6.813	388624	235927
13r2	6.804	403134	238437
14r1	6.804	383762	230998
14r2	6.804	389723	241084
15r1	6.633	1252482	280470
15r2	6.624	1197549	249190
16r1	6.597	703662	288658
16r2	6.597	655881	282806
17r1	6.57	325925	284788
17r2	6.579	341422	286665
18r1	6.57	249539	281257
18r2	6.579	260484	281991
19r1	6.579	410800	287402
19r2	6.588	482772	289631
20r1	6.588	377092	282448
20r2	6.57	377794	285634
21r1	6.651	567262	287010
21r2	6.651	574567	281342

Table 5: Comparison of internal standard areas and hydroxyacetaldehyde

2. Another reason for the lower detection of organic components from Biotox-16 to Biotox-21 was the higher amount of a quenching liquid detected in the gas chromatograms. This hydrocarbon liquid is used in the pyrolysis cooling system and leads to a dilution of the sample.

Figure 7 shows the results of quantified amount from pyrolytic lignin determination, water determination by Karl-Fischer and the GC-quantification. It also shows the GC unknowns which are

detectable by gas chromatography but could not be analyzed due to missing spectral and chromatographic data. Nevertheless, the most relevant peaks were quantified, as is shown in Figure 7. The data from Figure 7 are also presented in

Table 6.

It is also worthwhile to note that the amount of unknowns is larger in the sample set 1-15. This is because of the larger sample amount used for the preparation of the sample. As a consequence the amount of GC unknowns is increasing.
The large amount of aldehydes of Biotox-15 is due to an extraordinary amount of hydroxyl-acetaldehyde.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Py-Lignin	19.7	17.7	12.4	15.2	10.9	5.8	54.4	n/a	18.2	19.1	15.6	24.9	20.2	21.1	n/a	14.9	16.2	11.3	20.7	15.2	15.7
Water	23.5	28.5	29.1	24.9	29.4	37.0	8.1	28.6	26.8	22.4	26.7	20.3	24.6	22.7	12.1	18.2	25.5	31.8	23.0	26.8	17.6
GC known	22.3	21.5	26.9	24.5	27.5	22.0	21.6	25.1	26.9	25.7	27.8	28.1	28.7	27.8	31.6	19.6	21.8	19.1	15.3	19.9	19.1
GC unknown	11.6	9.9	9.4	10.4	8.9	7.7	13.5	10.5	8.8	9.6	7.7	11.1	12.1	10.6	5.6	4.9	1.5	2.3	1.3	1.6	1.2
Unknown portion	22.9	22.4	22.2	25.1	23.3	27.5	2.4	n/a	19.3	23.3	22.2	15.7	14.4	17.8	n7a	42.4	35.0	35.5	39.8	36.6	46.4

Table 6: Overall Composition of Biotox- oils

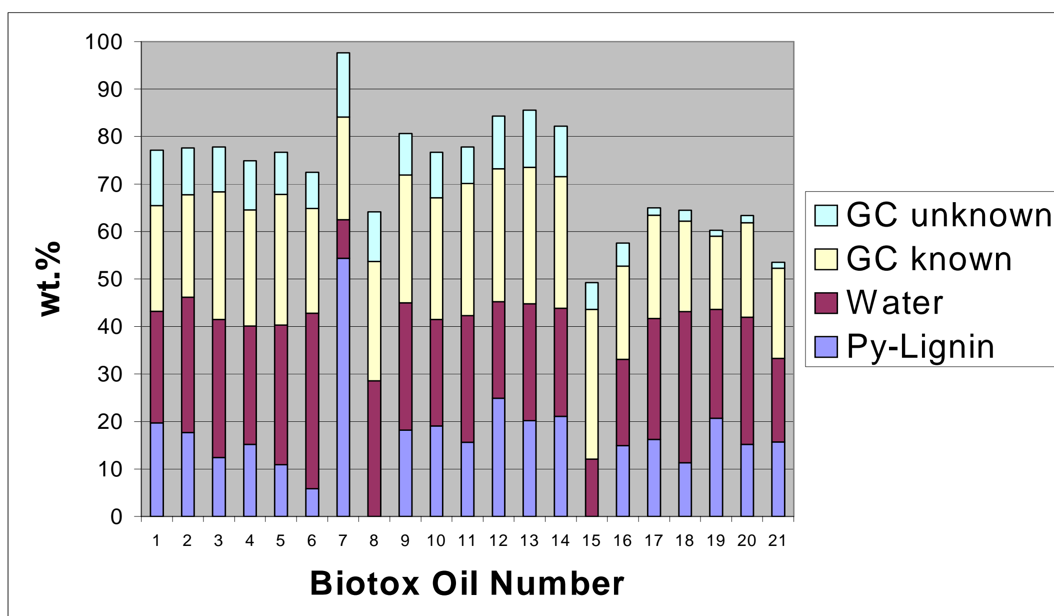

Figure 7: Quantified amounts of Biotox- samples

- Reproducibility of pyrolysis reaction and GC-analysis

In order to compare the pyrolytic conditions and the GC-results the following two figures were produced as Biotox-9 and Biotox-17 as well as Biotox-10 and Biotox-21 were produced under identical conditions.

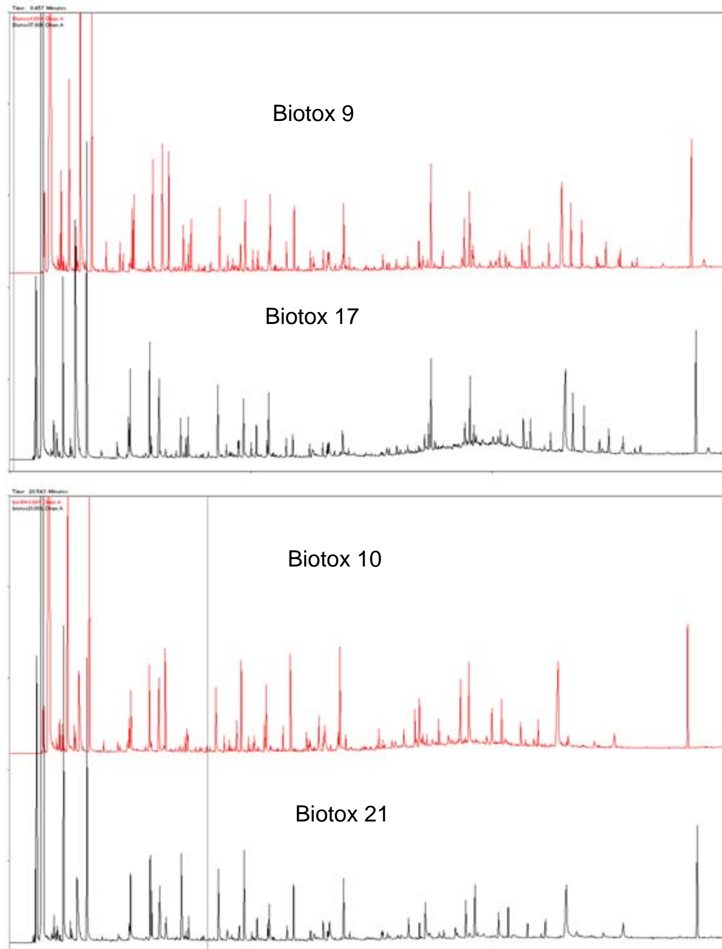

Figure 8: Comparison of Biotox-9 vs. Biotox-17 and Biotox-10 vs. Biotox-21

It is visible that Biotox-9 and Biotox-10 oils show more peaks which might be attributed to differences in the quenching system. Differences in peak intensity can be attributed to the more diluted samples Biotox-17 and Biotox-21 due to quenching liquid and the GC solvent acetone.

- PAH analysis

The existing PAH method of BFH was slightly modified in consultation with the EU JRC (Joint Research Centre, Geel). BFH has participated in a round robin test organized by JRC. The following
Table 7 gives the detailed steps for extraction and clean-up of PAH from pyrolysis oils.

- put 5 g pyrolysis oil in a beaker
- add 1 ml internal standard solution with benzo(A)anthracene D12, c = 4 µg/ml
- add 1 ml internal standard solution with anthracene D10, c = 4 µg/ml
- mix with 10 ml NaOH and pour into separation funnel
- extract with 60 ml cyclohexane
- remove NaOH phase
- add 10 ml NaOH
- extract; remove NaOH phase
- dry cyclohexane extract over Na_2SO_4
- condition 2 g Florisil-cartrige (MgO_3Si) with 15 ml cyclohexane
- add cyclohexane-extract on cartridge
- use 50 ml cyclohexane for elution
- add keeper (100 µl DMF) to eluate
- evaporate solvent to dryness
- dissolve in 1ml cyclohexane and fill vial for GC/MS

Table 7: Extraction and clean-up of PAH from pyrolysis oils

The results of the determination are presented in Table 8. Benzo(a)anthracene (BaA) and Benzo-(a)pyrene (BaP) are in bold as these components are the key substances for legislative limits. For comparison, in liquid smoke samples the limit for BaA is 0.01 ppm and for BAP is 0.02 ppm.

	Biotox 1 ppm	Biotox 2 ppm	Biotox 3 ppm	Biotox 4 ppm	Biotox 5 ppm	Biotox 6 ppm	Biotox 7 ppm
Fluorene	4.68	1.90	0.97	0.89	2.18	1.02	39.0
Phenantrene	4.54	1.64	0.76	0.46	2.53	2.09	23.8
Anthracene	1.08	0.27	0.17	0.15	0.79	0.54	14.0
Fluoranthene	0.77	0.42	0.21	0.11	0.36	0.50	6.15
Pyrene	1.38	0.58	0.23	0.18	0.75	0.65	9.53
Benzo(a)anthracene	**0.31**	**0.09**	**0.02**	**0.02**	**0.23**	**0.14**	**4.14**
Chrysene	0.27	0.09	0.05	0.03	0.15	0.14	3.16
Benzo(A)Fluoranthene	0.10	0.05	0.02	0.03	0.05	0.06	1.04
Benzo(K)Fluoranthene	0.09	0.03	0.01	0.03	0.05	0.07	1.06
Benzo(A)Pyrene	**0.24**	**0.07**	**0.03**	**0.04**	**0.16**	**0.12**	**1.32**
Indenopyrene	0.14	0.06	0.02	0.01	0.08	0.10	1.44
Dibenzoanthracene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Benzoperylene	0.11	0.06	0.02	0.02	0.06	0.10	n.d.
TOTAL	**13.71**	**5.28**	**2.52**	**1.97**	**7.40**	**5.53**	**104.55**

	Biotox 8 ppm	Biotox 9 ppm	Biotox 10 ppm	Biotox 11 ppm	Biotox 12 ppm	Biotox 13 ppm	Biotox 14 ppm
Fluorene	-	0.90	n.d.	n.d.	n.d.	n.d.	0.39
Phenantrene	-	1.07	0.83	3.70	9.63	n.d.	0.75
Anthracene	14.7	0.31	0.24	0.84	2.02	0.26	0.22
Fluoranthene	5.11	0.23	0.21	0.76	0.98	0.13	0.25
Pyrene	8.50	0.46	0.45	1.60	2.17	0.20	0.41
Benzo(a)anthracene	**2.16**	**0.15**	**0.19**	**0.81**	**1.68**	**0.03**	**0.16**
Chrysene	1.87	0.12	0.13	0.82	1.34	0.01	0.11
Benzo(A)Fluoranthene	0.51	0.06	0.08	0.37	0.78	n.d.	0.08
Benzo(K)Fluoranthene	0.41	0.06	0.07	0.39	0.67	n.d.	0.08
Benzo(A)Pyrene	**n.d.**	**0.17**	**0.20**	**0.97**	**1.88**	**0.04**	**0.19**
Indenopyrene	0.37	0.08	0.16	0.43	0.98	n.d.	0.12
Dibenzoanthracene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Benzoperylene	0.35	0.08	0.13	0.58	1.28	0.03	0.09
TOTAL	**33.95**	**3.70**	**2.70**	**11.28**	**23.43**	**0.70**	**2.86**

	Biotox 15 ppm	**Biotox 16** ppm	**Biotox 17** ppm	**Biotox 18** ppm	**Biotox 19** ppm	**Biotox 20** ppm	**Biotox 21** ppm
Fluorene	5.03	0.03	n.d.	2.81	0.66	1.23	0.13
Phenantren	5.30	0.05	0.10	0.97	1.26	0.85	0.21
Anthracene	1.40	0.01	0.05	0.29	0.32	0.23	0.06
Fluoranthene	0.17	n.d.	0.01	0.35	0.31	0.23	0.06
Pyrene	1.24	0.02	0.06	0.35	0.63	0.35	0.26
Benzo(a)anthracene	**0.50**	**n.d.**	**0.02**	**0.06**	**0.23**	**0.11**	**0.03**
Chrysene	0.36	n.d.	0.01	0.10	0.18	0.09	0.02
Benzo(A)Fluoranthene	0.19	n.d.	0.01	0.03	0.09	0.04	0.01
Benzo(K)Fluoranthene	0.17	n.d.	0.01	0.01	0.08	0.03	0.01
Benzo(A)Pyrene	**0.84**	**0.17**	**0.39**	**0.47**	**0.57**	**0.49**	**0.30**
Indenopyrene	0.15	n.d.	0.01	0.03	0.09	0.04	0.01
Dibenzoanthracene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Benzoperylene	0.09	n.d.	0.01	0.03	0.08	0.04	0.02
TOTAL	**10.41**	**0.25**	**0.70**	**2.68**	**3.84**	**2.50**	**1.01**

Table 8: Determination of most relevant PAH's in bio-oils

A visual inspection of the PAH composition is shown in Figure 9. It is clearly demonstrated that the Biotox-7 and Biotox-8 oils have the highest amounts of PAH. This was expected and is due to the relatively long pyrolysis times compared to the other fast pyrolysis processes.

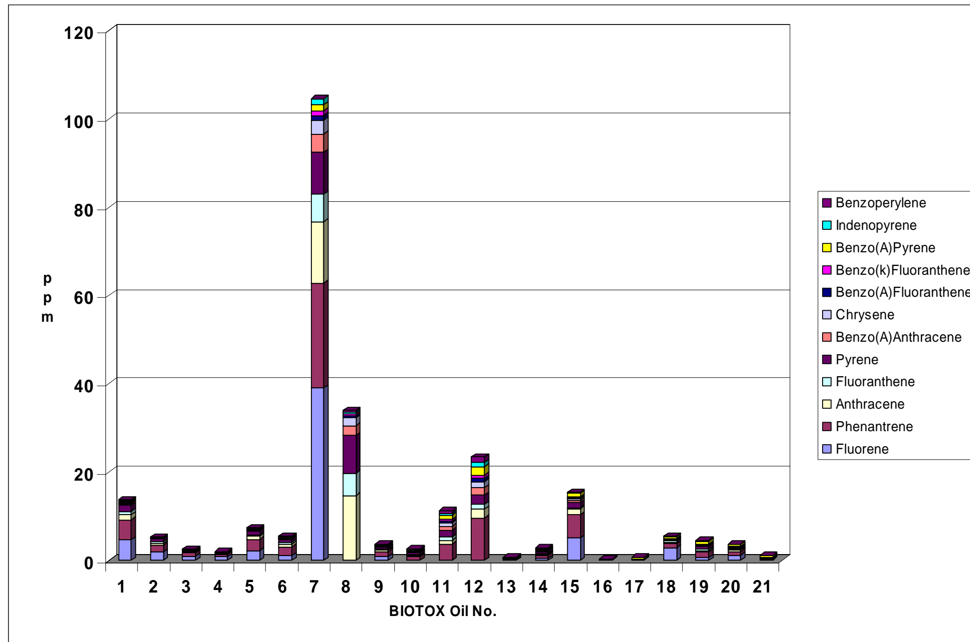

Figure 9: Graphical comparison of PAH's amounts and composition

In order to improve visualization of the smaller remaining PAH data in Figure 9, Figure 10 was produced excluding the data set of Biotox-7 and Biotox-8.

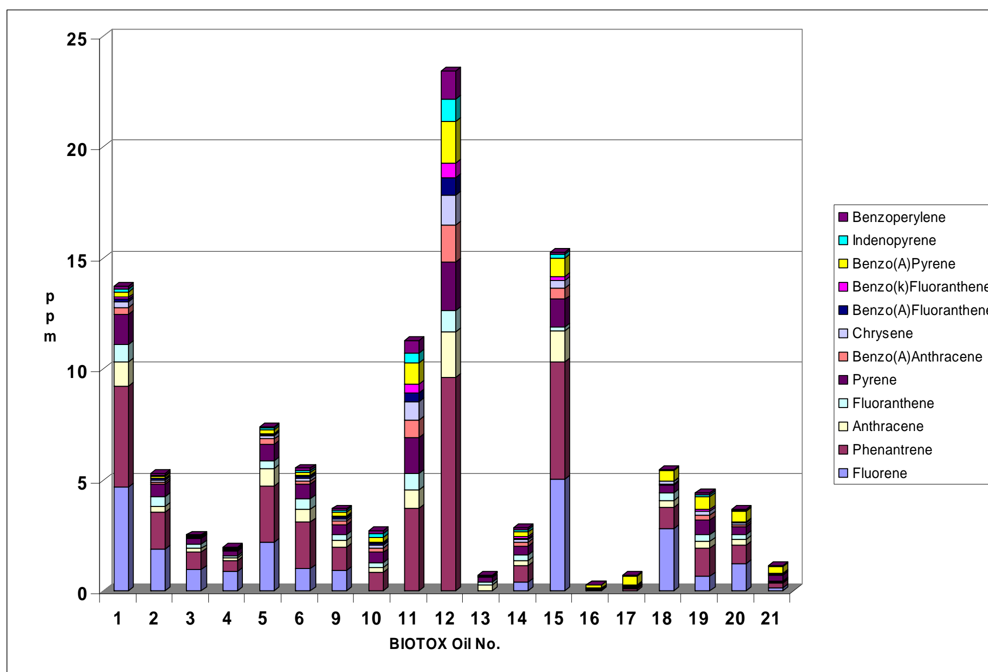

Figure 10: Graphical comparison of PAH's amounts and composition with exclusion of Biotox-7 and Biotox-8

As Benzo(a)pyrene is considered the most toxic component it amount in the different Biotox-samples is illustrated in Figure 11.

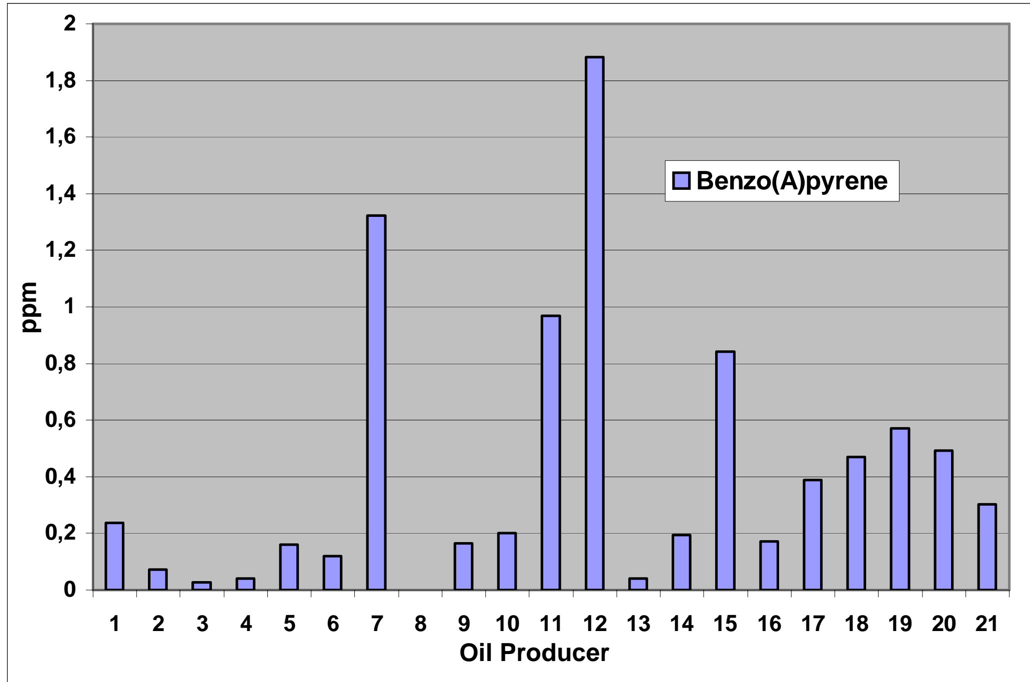

Figure 11: Benzo(a)pyrene content in Biotox oils

Interestingly, Biotox-11 and Biotox-12 show relative high values although they are produced by fluidized bed pyrolysis. Biotox-12 shows the highest amount as is was produced at 600°C.

-UV/VIS-Spectral Analysis

Because some of the CIT laboratory test reported on possible effects of light absorption, the spectra in the ultra violet region (190-400 nm) and the visible light region (400-850 nm) were recorded with a spectralphotometer. The overall spectra for the whole wavelength region are presented in Figure 12.

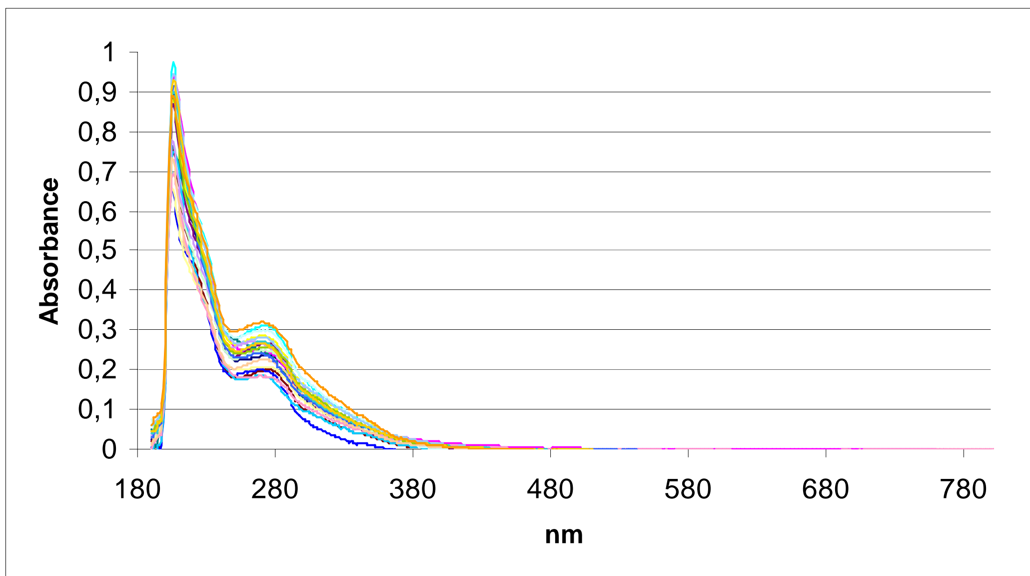

Figure 12 : UV/VIS spectra of all 21 Biotox samples

No important differences can be observed. Figure 13 shows the UV wavelength region. Again, no significant differences can be observed. The maximum at 200 nm results from the absorption of

carbohydrate derived components and is not very specific. The smaller maximum at 280 nm can be assigned to aromatic structures.

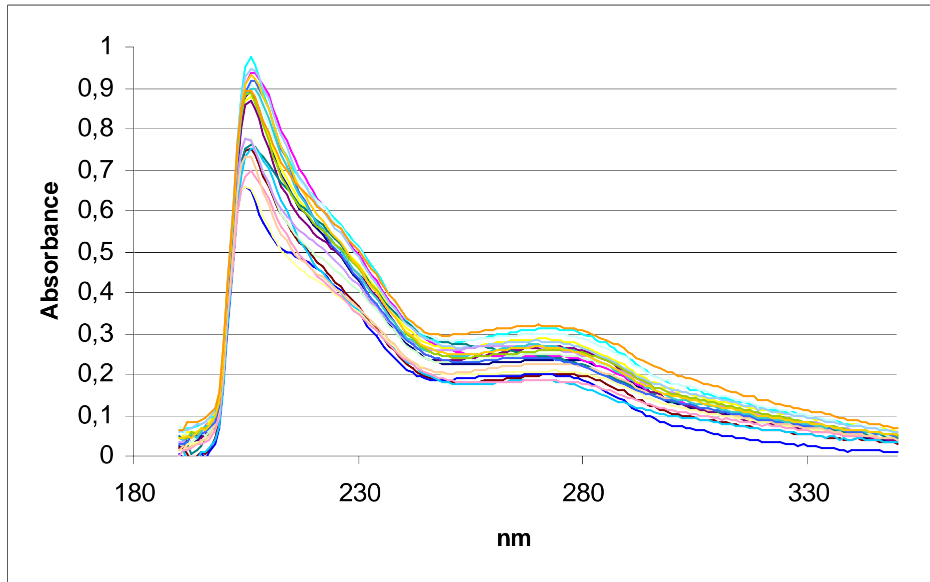

Figure 13: UV wavelength region of all 21 Biotox samples

b) Correlations between oil properties and production processes

Correlations can only be done from oil made at Aston as all oils were produced with the same equipment under controlled conditions.

* Nature of feedstock

Feedstock comparison is possible between Biotox-9 (beech wood) and 10 (spruce wood). The water content is rather similar. The spruce oil is has a 3 fold higher viscosity.

* Temperature

The effect of temperature can be observed using Biotox-11 (400°C), Biotox- 10 (500°C), Biotox-15 (575°C), and Biotox-12 (600°C). Water is indifferent. Viscosity is higher at 600°C, pyrolytic raises with temperature from 15 to 25 %, and the carbon content raises with temperature from 39 to 45 %.

* Aging

Aging can be discussed by comparing Biotox-19 (12 months old) vs. Biotox-21. As expected the water content of Biotox-19 is 5 % higher than that of Biotox-21 due to possible recondensation reactions during storage. Surprisingly, the viscosity is slightly higher with the "young" oil. The pyrolytic lignin amount is also 5 % higher with the old oil and corroborates results of other studies.

I.2.2.2 Bio-oils Toxicological screening tests

As controversial data concerning the bio-oil's mutagenicity were published in the few toxicological studies reported in the literature the project steering comity decided to assess the potential mutagenic effect of Bio-oils, via the "Bacterial reverse mutation test"

The objective of this study is to evaluate the potential of bio-oils to induce reverse mutation in *Salmonella typhimurium*. The bacterial reverse mutation test is able to identify substances that cause point mutations, by substitution, addition or deletion of one or a few DNA base-pairs. Mutagenic substances can induce reversion in histidine deficient strains, which are then able to grow and form colonies in a histidine-limited medium, while non-reverted strains cannot. This test is performed in the absence and presence of a rat liver metabolizing system (S9 mix).
Guideline: Commission directive 2000/32/EC,B13,8 June 2000 and OECD Guideline No. 471, 21st July 1997

a) Preparation of the test solutions
The vehicle for solubilising bio-oil was selected according to results from solubility trials performed before the preliminary toxicity test. Dimethylsulfoxide (DMSO) appeared mainly to be the best vehicle for bio-oil.

b) Bacterial strains
Five strains of *Salmonella typhimurium* are used to carry out this study: TA 1535, TA 1537, TA 98, TA 100 and TA 102. The day before treatment, cultures are inoculated from bacterial suspensions. Each strain derived from *Salmonella typhimurium* LT2 contains one mutation in the histidine operon, resulting in a requirement for histidine.

c) Metabolic activation system
The S9 mix consists of induced enzymatic systems contained in rat liver post-mitochondrial fraction (S9 fraction) and the co-factors necessary for their function.

d) Mutagenicity experiment
In one mutagenicity experiment, using three plate/dose-level, each strain are tested, with and without S9 mix, with:

- at least five dose-levels of bio-oil,
- the vehicle control,
- the appropriate positive control

e) Evaluation of the results
In each experiment, for each strain and for each experimental point, the number of revertants per plate is scored. Studies are considered valid if the following criteria are fully met:

- the number of revertants in the vehicle controls is consistent with historical data,
- the number of revertants in the positive controls is higher than that of the vehicle controls and is consistent with historical data.

A 2-fold increase (for the TA 98, TA 100 and TA 102 strains) or 3-fold increase (for the TA 1535 and TA 1537 strains) in the number of revertants compared with the vehicle controls, in any strain at any dose-level and/or evidence of a dose-relationship is considered as a positive result.

f) *Results*

Obtained results are recapitulated in following Table 9.

Biotox	S9 MIX	µg/ plate	TA 1537	TA 1535	TA 98	TA 100	TA 102
Biotox 1	-	5000	(yellow)			(red)	
Biotox 1	-	2500					T (red)
Biotox 1	-	2000		T (yellow)	T (yellow)		
Biotox 1	+	5000	(yellow)			(red)	(orange)
Biotox 1	+	2500		T (yellow)	T (orange)		
Biotox 2	-	5000	(yellow)			(orange)	(red)
Biotox 2	-	4000		T (red)			
Biotox 2	-	2000			T (yellow)		
Biotox 2	+	5000	(yellow)	(yellow)	(red)	(orange)	(orange)
Biotox 3	-	5000	(yellow)			(red)	(red)
Biotox 3	-	4000		T (yellow)			
Biotox 3	-	2000				T (orange)	
Biotox 3	+	5000	(yellow)	(yellow)	(red)	(red)	(red)
Biotox 4	-	4000				T (red)	T (red)
Biotox 4	-	2000	T (yellow)	T (yellow)	T (yellow)		
Biotox 4	+	5000	(yellow)			(red)	(red)
Biotox 4	+	4000		T (yellow)			
Biotox 4	+	2000			T (red)		
Biotox 5	-	5000	(yellow)	(red)	(red)	(red)	(red)
Biotox 5	+	5000	(red)	(yellow)	(red)	(red)	(red)
Biotox 6	-	5000	(yellow)			(red)	(red)
Biotox 6	-	2500		T (yellow)	T (red)		
Biotox 6	+	5000	(yellow)			(red)	(red)
Biotox 6	+	2500		T (red)	T (red)		
Biotox 7	-	2500					P (yellow)
Biotox 7	-	625	T (yellow)			T (yellow)	
Biotox 7	-	156,3		T (yellow)	T (yellow)		
Biotox 7	+	2500	P (yellow)	T (yellow)	P/T (orange)	P (yellow)	P (yellow)
Biotox 8	-	2500		T (yellow)	T (red)		T (yellow)
Biotox 8	-	1250	T (yellow)			T (yellow)	
Biotox 8	+	5000				T (yellow)	
Biotox 8	+	2500		T (yellow)	T (red)		T (yellow)
Biotox 8	+	1250	T (yellow)				
Biotox 9	-	3000	T (yellow)			T (orange)	T (orange)
Biotox 9	-	1000			T (orange)		
Biotox 9	-	250		T (yellow)			
Biotox 9	+	5000	(yellow)			(red)	(red)
Biotox 9	+	3000		T (yellow)	T (yellow)		
Biotox 10	-	2500	T (yellow)			T (red)	
Biotox 10	-	1875					T (orange)
Biotox 10	-	1250		T (yellow)	T (red)		
Biotox 10	+	5000	(yellow)			(red)	(red)
Biotox 10	+	2500		T (yellow)	T (red)		
Biotox 11	-	5000	(yellow)			(red)	(red)
Biotox 11	-	2500		T (yellow)	T (red)		
Biotox 11	+	5000	(yellow)	(yellow)	(red)	(red)	(red)
Biotox 12	-	5000	(yellow)			(red)	
Biotox 12	-	3750					T (red)
Biotox 12	-	1875		T (yellow)	T (red)		
Biotox 12	+	5000	(yellow)			(red)	(red)
Biotox 12	+	2500		T (red)	T (red)		
Biotox 13	-	5000	(yellow)			(red)	(yellow)
Biotox 13	-	3750		T (yellow)	T (yellow)		
Biotox 13	+	5000	(yellow)			(red)	
Biotox 13	+	3750		T (yellow)	T (yellow)		T (red)
Biotox 14	-	3750	T (yellow)		T (red)	T (red)	T (orange)
Biotox 14	-	2500		T (yellow)			
Biotox 14	+	3750	T (yellow)	T (orange)	T (yellow)	T (red)	T (red)
Biotox 15	-	5000					T (red)
Biotox 15	-	3750	T (yellow)	T (yellow)	T (red)	T (yellow)	
Biotox 15	+	5000	T (yellow)	T (yellow)	T (yellow)	T (red)	T (red)
Biotox 16	-	3750	T (yellow)			T (yellow)	T (red)
Biotox 16	-	1250		T (yellow)	T (red)		
Biotox 16	+	5000					T (red)
Biotox 16	+	3750	T (yellow)	T (yellow)	T (red)	T (red)	
Biotox 17	-	5000					T (red)
Biotox 17	-	2500	T (yellow)	T (yellow)	T (red)	T (yellow)	
Biotox 17	+	5000					T (red)
Biotox 17	+	3750	T (yellow)	T (yellow)	T (red)	T (orange)	
Biotox 18	-	5000	T (yellow)	T (yellow)	T (red)	T (red)	T (red)
Biotox 18	+	5000	(yellow)	T (yellow)	T (orange)	T (yellow)	(red)
Biotox 19	-	3750	P/T (yellow)	P/T (yellow)	P/T (yellow)	P/T (orange)	P (orange)
Biotox 19	+	5000	P/T (yellow)	P/T (yellow)	P/T (red)	P (orange)	P (red)
Biotox 20	-	5000					T (red)
Biotox 20	-	3750	T (yellow)	T (yellow)	T (orange)	T (yellow)	
Biotox 20	+	5000	(yellow)	T (yellow)	T (red)	T (yellow)	T (red)
Biotox 21	-	1250	T (yellow)	T (yellow)	T (red)	T (yellow)	T (red)
Biotox 21	+	3750					T (red)
Biotox 21	+	1250	T (yellow)	T (yellow)	T (red)	T (red)	

Doses tested (µg/plate), S9 MIX -: 5000, 4000, 3750, 3000, 2500, 2000, 1875, 1250, 1000, 625, 250, 156,3
Doses tested (µg/plate), S9 MIX +: 5000, 4000, 3750, 3000, 2500, 2000, 1875, 1250, 1000, 625, 156,3

Coloured compartment : the strongest bio-oil dose tested in the study
When the strongest tested dose< 5000 µg/fla P for precipitation and\or T for toxicity in preliminary toxicity tests

(red) result clearly positive (mutagenic effect). (yellow) no mutagenic effect was noted
(orange) ambiguous result with increase of the number of revertants, which seems to depend on the bio-oil dose level tested.

Table 9: Results of the mutagenicity ames tests

In Table 9 for each oil, each strain, with or without S9 mix, the highest concentration tested in the study is marked by a coloured compartment.

Lower concentration than 5000 µg/flat might have been used when :

- the solubility of the bio oil is limiting the concentration labelled by a P (precipitation)
- the bio-oil is too toxic for the test to be representative labelled by a T (toxic)

Levels of concentration to be used are assessed by preliminary toxicity test.

Compartments are yellow if no mutagenic effect was noted, red colour is used if the result was clearly positive (mutagenic effect). Compartments coloured in orange corresponded to ambiguous result. In those cases an increase of the number of revertants was observed, which seem to depend on the bio-oil dose level tested. But the increased in number of revertants compared with the vehicle controls didn't reach the level specified in international regulations to consider a positive mutagenic result. The observation of a dose-relationship being rather subjective; additional tests would be necessary to verify the reproducibility.

For all bio-oils, at least one test for one Salmonella typhimurium strain, the increase in number of revertants reach the level specified in international regulations to consider a positive mutagenic result (red colored compartment). Base on these results all bio-oils are considered to be mutagenic.
Some oils seem to be more mutagenic than others, but it is not possible to compare the oils mutagenicity as the tested concentrations are different. Indeed, in a lot of cases highest oils concentrations tested are below 5000 µg/flat, because in preliminary toxicity tests those samples appeared to be toxic or not soluble at high concentrations. In such cases the toxicity and/or the low solubility of the sample forbid mutagenicity assessment at high concentration.
However it emerges that oils produced by slow pyrolysis (Biotox-7 & -8) behave differently from the other oils. They have a strongly toxic effect on the strains. It limits the study of their mutagenicity while, in order to minimize their toxic effect, it is necessary to work on very weak bio-oils dose level.
Interpretations of these results are not easy. It is in fact not possible to compare bio-oils between themselves as tested dose levels are variables.
However, it seems that for all these tests the number of revertants generally increases when the quantity of oil increased, up to a certain oil concentration from which the toxicity of oil prevents the development of reverted bacteria.

I.2.2.3 Bio-oils Ecotoxicological screening tests

In order to assess how pyrolysis oils could affect the environment and the organisms living in it, ecotoxicological screening tests were carried on with the 21 samples. Ecotoxicology is the basis for defining the No-Effect Loading Rate (NOEL) used in risk assessment. The potential ecotoxicity of bio-oils was studied on unicellular algal (Algal growth inhibition test) and on small animal (Acute toxicity in Daphnia magna STRAUS).

a) Algal growth inhibition test

The objective of this study is to assess the effects of bio-oils on the growth of an unicellular green algal species *Scenedesmus subspicatus* (a rapidly growing unicellular species), in a 72-hour static test. The criterion measured is the EL50 (Medium Effect Loading rate), a statistically derived loading rate of the bio-oil in water which can be expected to cause a reduction of growth or growth rate of 50% in the treated algal populations relative to the control.

This study has been designed to comply with the following guideline

Guidelines	Commission directive 92/69/EEC, C.3, 31st July 1992 OECD Guideline No. 201, 7th June 1984
Species	*Scenedesmus subspicatus* *Pseudokirchneriella subcapitata*
Study design	Control + generally 3 concentrations: 1, 10 and 100 mg/L The WAF methodology is proposed for poorly soluble substances (solubility < 100 mg/L
Results	Growth rate inhibition: estimation of the EC50 after 72 hours Biomass inhibition: estimation of the EC50 after 72 hours

Table 10: Informations about the algal growth inhibition test

Test system

Tests were carried out by subjecting three replicates of algal culture to bio-oils test solutions (loading rates of bio-oil 1, 10 ad 100 mg/l), over a 72-hours period. The number of cells were counted at each observation time (T24, T48 and T72 hours) using a Cell Counter.
The criterion measured was the EL50 (Medium Effect Loading rate), a statistically derived loading rate of the bio-oil in water which can be expected to cause a reduction of growth or growth rate of 50% in the treated algal populations relative to the control.:

Data evaluation

The calculated percentages of inhibition of the cell growth rate and growth at each loading rate of bio-oil tested are determined following the procedures recommended in EEC and OECD guidelines.
The percentage inhibition of the growth rate or growth is based on comparison between the growth rate or growth for each loading rate and the growth rate or growth for the control. This comparison allows to determine respectively an ErL50 or EbL50 - loading rate of the test item resulting in 50% reduction of the specific growth rate or growth with respect to the control.

Where possible, the 72-hour ErL 50 and the 72-hours EbL 50 are estimated as follows:

- EL50 ≤ 1 mg/L
- or 1 < EL50 ≤ 10 mg/L
- or 10 < EL50 ≤ 100 mg/L
- or EL50 > 100 mg/L

For the test to be valid, the cell concentration in the control cultures should have increased by a factor of at least 16 within three days.

b) Acute toxicity in daphnia magna

The objective of this study is to assess the acute toxicity of bio-oil in *Daphnia magna* STRAUS – clone 5 (the most sensitive clone of the species), in a 48-hour static test. Due to the small size of the Daphnia, mortality is difficult to determine and therefore the test criterion of acute toxicity used is immobilization, expressed as EC50 (Median Effective Concentration, a statistically derived concentration of the test item (bio-oil) in water at which 50% of animals are immobilized). An animal is defined as immobile when it is incapable of swimming within 15 seconds after gentle agitation of the test container. The criterion measured is the EL50 (Median Effect Loading rate), a statistically derived loading rate of the test item (bio-oil) in water at which 50% of animals are immobilized.
This study has been designed to comply with the following guidelines:

Guidelines	Commission directive 92/69/EEC,C.2, 31st July 1992 OECD Guideline No. 202, 4th April 1984
Species	*Daphnia magna STRAUS, clone 5*
Study design	Control + generally 3 concentrations: 1, 10 and 100 mg/L The WAF methodology is proposed for poorly soluble substances (solubility < 100 mg/L)
Results	Immobilization: estimation of the EC50 after 24 and 48 hours

Table 11: Informations about the Acute toxicity in daphnia magna

Test system

Daphnia (animals) are selected without preference and randomly assigned to test vessels. The test is carried out by subjecting 20 Daphnia to a maximum loading rate of 100 mg/l and further groups of 20 Daphnia to loading rates of 1 and 10 mg/l, over a 48-hour period. The control also contains 20 Daphnia.

The test is carried for 48 hours and immobilisation is noted at T0, T24 and T48 hours by gently shaking the test vessel. If an animal is incapable of swimming within 15 seconds after it has been disturbed, it is considered immobile.

Data evaluation

Where possible, the 24 and 48-hour EL50 are estimated as follows:

- EL50 ≤ 1 mg/L
- or 1 < EL50 ≤ 10 mg/L
- or 10 < EL50 ≤ 100 mg/L
- or EL50 > 100 mg/L

For a test to be valid, the following conditions should be fulfilled:

- The immobilisation in the control should not exceed 10% at the end of the test;
- The dissolved oxygen concentration should remain ≥ 60% of the air saturation value throughout the test.

c) Results of the ecotoxicological tests:

Obtained results for the two tests reported in Table 12 and Table 13.

The 21 pyrolysis-oils can be shared in three categories:

1. 12 bio-oils with no eco-toxicology (Biotox -3; 5; 6; 7; 11; 12; 14; 16; 18; 19; 20; 21);
2. 8 bio-oils with very light eco-toxicology (Biotox -1; 2; 4; 9; 10; 13; 15; 17);
3. and one eco-toxic pyrolysis oils (Biotox 8 slow pyrolysis sample)

TEST ITEM	STUDY	RESULTS
BIOTOX-3	Algal growth inhibition test	The 0-72h ErL50 was > 100 mg/L The 0-72h EbL50 was > 100 mg/L The 0-72h NOEL was ≥ 100 mg/L
	Acute toxicity in *Daphnia magna*	The 48h EL50 was > 100 mg/L The 0-48h NOEL was ≥ 100 mg/L
BIOTOX-5	Algal growth inhibition tes	**Like BIOTOX-3**
	Daphnia magna	” ” ” ”
BIOTOX-6	Algal growth inhibition test	” ” ” ”
	Acute toxicity in *Daphnia magna*	” ” ” ”
BIOTOX-7	Algal growth inhibition test	” ” ” ”
	Acute toxicity in *Daphnia magna*	” ” ” ”
BIOTOX-11	Algal growth inhibition test	” ” ” ”
	Acute toxicity in *Daphnia magna*	” ” ” ”
BIOTOX-12	Algal growth inhibition test	” ” ” ” Significant inhibition of algal growth by light absorption at 10 and 100 mg/L
	Acute toxicity in *Daphnia magna*	” ” ” ”
BIOTOX-14	Algal growth inhibition test	The 0-72h ErL50 was > 100 mg/L The 0-72h EbL50 was > 100 mg/L The 0-72h NOEL was 10 mg/L
	Acute toxicity in *Daphnia magna*	The 48h EL50 was > 100 mg/L The 0-48h NOEL was 10 mg/L
BIOTOX-16	Algal growth inhibition test	” ” ” ” No significant inhibition of algal growth by light absorption up to 100 mg/L
	Acute toxicity in *Daphnia magna*	” ” ” ”
BIOTOX-18	Algal growth inhibition test	” ” ” ” No significant inhibition of algal growth by light absorption up to 100 mg/L
	Acute toxicity in *Daphnia magna*	” ” ” ”
BIOTOX-19	Algal growth inhibition test	” ” ” ” Significant inhibition of algal growth by light absorption at 100 mg/L
	Acute toxicity in *Daphnia magna*	” ” ” ”
BIOTOX-20	Algal growth inhibition test	The 0-72h ErL50 was > 100 mg/L The 0-72h EbL50 was > 100 mg/L The 0-72h NOEL was 10 mg/L No significant inhibition of algal growth by light absorption up to 100 mg/L
	Acute toxicity in *Daphnia magna*	” ” ” ”
BIOTOX-21	Algal growth inhibition test	” ” ” ” No significant inhibition of algal growth by light absorption up to 100 mg/L
	Acute toxicity in *Daphnia magna*	” ” ” ”

Table 12: Pyrolysis oils without eco-toxicity

LOWEST EL50 CLOSE TO 100 MG/L		
BIOTOX-4	Algal growth inhibition test	0-72h ErL50 was > 100 mg/L **0-72h EbL50** estimated close to 100 mg/L 0-72h NOEL was 10 mg/L
	Acute toxicity in *Daphnia magna*	48h EL50 was > 100 mg/L 0-48h NOEL was ≥ 100 mg/L
BIOTOX-9	Algal growth inhibition test	0-72h ErL50 was > 100 mg/L **0-72h EbL50** to be close to 100 mg/L 0-72h NOEL was 10 mg/L
	Acute toxicity in *Daphnia magna*	48h EL50 was > 100 mg/L 0-48h NOEL was ≥ 100 mg/L
BIOTOX-13	Algal growth inhibition test	0-72h ErL50 was > 100 mg/L **0-72h EbL50** was estimated close to 100 mg/L 0-72h NOEL was 10 mg/L No inhibition by light absorption up to 100 mg/L
	Acute toxicity in *Daphnia magna*	48h EL50 > 100 mg/L 0-48h NOEL was 10 mg/L
BIOTOX-17	Algal growth inhibition test	0-72h ErL50 > 100 mg/L **0-72h EbL50** estimated close to 100 mg/L 0-72h NOEL was 10 mg/L No inhibition by light absorption up to 100 mg/L
	Acute toxicity in *Daphnia magna*	48h EL50 was > 100 mg/L 0-48h NOEL was ≥ 100 mg/L

LOWEST EL50 BETWEEN 10 AND 100 MG/L		
BIOTOX-1	Algal growth inhibition test	0-72h ErL50 was > 100 mg/L **0-72h EbL50** estimated between 10 and 100 mg/L 0-72h NOEL was 10 mg/L
	Acute toxicity in *Daphnia magna*	48h EL50 was > 100 mg/L 0-48h NOEL was ≥ 100 mg/L
BIOTOX-2	Algal growth inhibition test	0-72h ErL50 was > 100 mg/L **0-72h EbL50** between 10 & 100 mg/L 0-72h NOEL was 10 mg/L
	Acute toxicity in *Daphnia magna*	48h EL50 was > 100 mg/L 0-48h NOEL was ≥ 100 mg/L
BIOTOX-15	Algal growth inhibition test	0-72h ErL50 to be close to 100 mg/L **0-72h EbL50** between 10 & 100 mg/L. 0-72h NOEL was 10 mg/L No inhibition by light absorption up to 100 mg/L
	Acute toxicity in *Daphnia magna*	48h EL50 was > 100 mg/L 0-48h NOEL was 10 mg/L
LOWEST EL50 CLOSE TO 10 MG/L		
BIOTOX-10	Algal growth inhibition test	0-72h ErL50 > 100 mg/L **0-72h EbL50** estimated close to 10 mg/L 0-72h NOEL was < 1 mg/L
	Acute toxicity in *Daphnia magna*	48h EL50 was > 100 mg/L 0-48h NOEL was ≥ 100 mg/L
LOWEST EL50 BETWEEN 1 AND 10 MG/L		
BIOTOX-8	Algal growth inhibition test	0-72h ErL50 between 10 and 100 mg/L **0-72h EbL50 between 1 and 10 mg/L** 0-72h NOEL was 1 mg/L
	Acute toxicity in *Daphnia magna*	48h EL50 between 10 and 100 mg/L The 0-48h NOEL was 10 mg/L

Table 13: Pyrolysis oils light eco-toxicity (except Biotox 8)

Based on these obtained results, it comes into view that fast pyrolysis oils have none or weak eco-toxicological effect.
The Acute toxicity in daphnia magna study demonstrates that (except the slow pyrolysis oil sample Biotox 8) bio-oils have not toxicological effect on small animals.
The Algal growth inhibition study demonstrated a very rare effect of flash pyrolysis oils on the unicellular green algal. Even low concentrations of bio-oils in the medium had a fertilizer effect, increasing algal growth.
This inhibition of algal growth can be due either to a toxic effect of the oil sample or by light adsorption which reduces photosynthesis activity (bio-oils are dark black and can slow down light diffusion).
In order to determine the importance of both phenomena, further light absorption tests were performed on samples Biotox -12; 13; 15; 16; 17, 18; 19; 20; 21. These tests consisted of studying the algal growth in different light filtered area, with and without bio-oils.
Results obtained for Biotox -12 and -21 shown that samples absorbed a significant amount of light. While results obtained for other sample shown that a growth inhibition didn't come from light absorption, and can only be due to a toxic effect of the oil. It seems that both effects (toxicity and light absorption) induce the growth observed inhibition effect, and according to samples these phenomena can be more or less important

I.2.2.4 Bio-oils Aerobic biodegradability in fresh water

The aim of the study is to measure the aerobic biodegradability of pyrolysis oils in order to evaluate if they could be a local environmental hazard in case of accidental discharges.

As bio-oils have a low solubility in water and the volatility of some of their components, analyse of their biodegradability was difficult. It was necessary in a first time to experiment and to adapt tests protocols to measure the biodegradability of bio-oils..

a) Methodology

The bio-oils biodegradability was assessed based on the OECD 301B modified Sturm Test. This test is applied to a substance that has passed a stringent test for ultimate biodegradability, which is internationally accepted as reference test. It was developed to identify substances that would be rapidly and extensively biodegraded in the aqueous environment and it's stringency lead to good estimation of an oil product's biodegradability.

Test Method	Official OECD "ready test" procedures 301B Sturm Modified; Respirometry CO_2 evolution
Inoculum	Activated sludge from sewage treatment plant (32 mg/l SS)
Bio-oils concentrations	15 mg/l Dissolved Organic Carbon
Number of flasks	Samples, reference substance, and blanks are duplicates
Reference Substance	Sodium acetate
Measures	CO_2 is trapped in $Ba(OH)_2$ and is measured by titration of the residual OH^-
Blank values	$CO_2 < 40$ mg/l
Test duration	28 days

Table 14: Informations about the biodegradability modified Sturm test

b) Test system

A controlled volume of inoculated mineral medium, containing a known concentration of the tested substance (10-20 mg DOC/l) as the nominal sole source of organic carbon is aerated by a controlled flow of carbon dioxide-free air in the dark or in diffuse light conditions. Degradation is followed over 28 days by determining the carbon dioxide produced. The CO_2 is trapped in barium hydroxide and is measured by titration of the residual hydroxide. The amount of carbon dioxide produced from the test substance (corrected for that derived from the blank inoculum) is expressed as a percentage of theoretical carbon dioxide ($ThCO_2$). The test lasts for 28 days

Only 15 mg/l of dissolved organic Carbon from bio-oils sample had to be added to the inoculated mineral solution. As bio-oils have a high carbon content, Cirad developed a method to easily weigh the small amounts of sample to be assessed. Samples were weighed on a non-biodegradable solid carrier (glass filter).

All tests were performed in replicates and obtained values are close (average deviation between 0,5 and 2,4 %).

c) Results

In addition to the 21 oils samples, a conventional diesel oil sample was tested for comparison. Biodegradability curves measured over the 28 days are presented in following Figure 14.

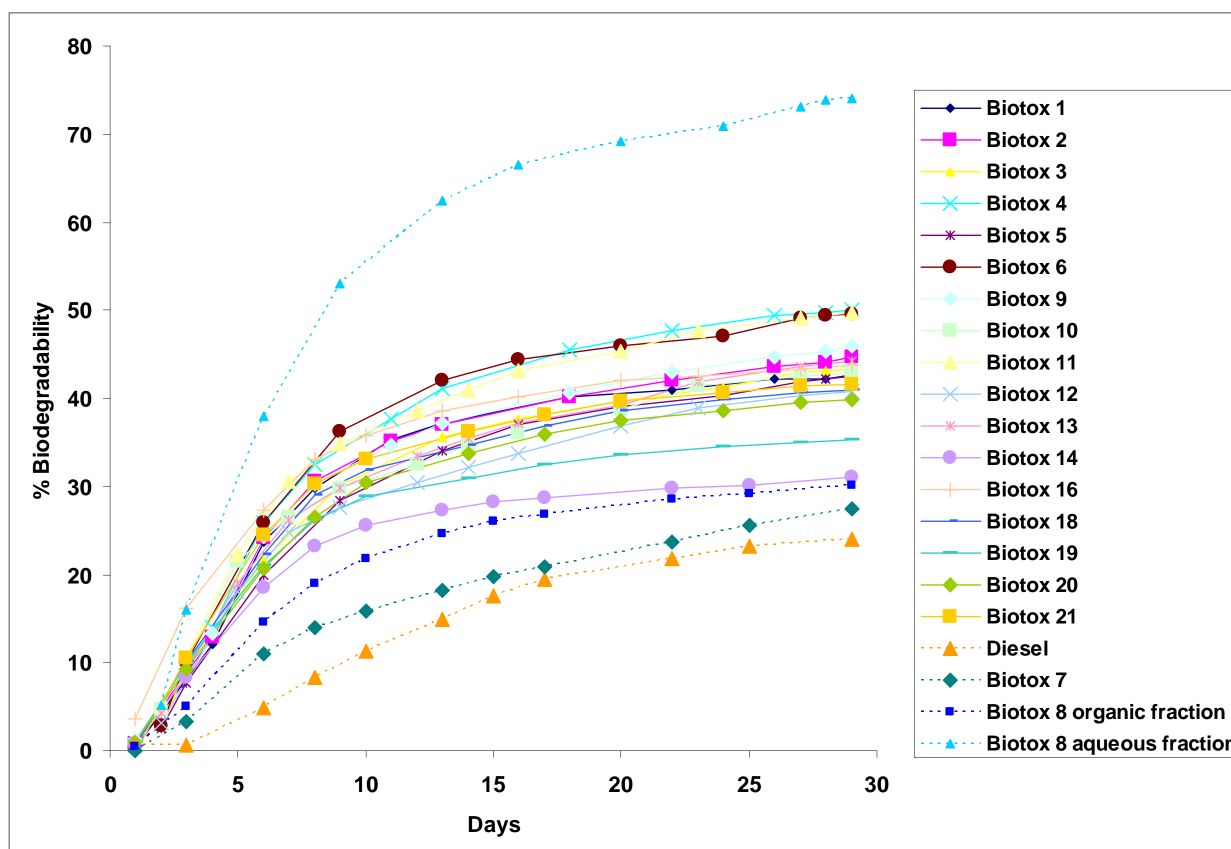

Figure 14: Biodegradation curves of pyrolysis oils following the 301B OECD test method.

Biodegradation reactions were described by a simple first-order kinetic Eq. (1) and regression-processed by the least squares method, minimization of target function applying the Solver subprogram of Microsoft Excel 7.0.

$$D_{CO_2} = D_{max}\left(1 - e^{-k(t-t_{lag})}\right) \quad \text{Eq. (1)}$$

where:

D_{CO2} , D_{max},	biodegradation in time t, and maximal (limit) value
k	rate constant (h^{-1})
t_{lag}	lag phase of biodegradation (days).

Kinetic data are numerically presented in Table 15.

	Measured	Calculated			
Bio-oils	**D_{CO2} (28 days)**	**Dmax (%)**	**k ($days^{-1}$)**	**$t_{(lag)}$ (days)**	**$t_{1/2}$ (days)**
Biotox - 1	42,66	43,23	0,16	1,14	4,40
Biotox - 2	44,64	44,75	0,15	1,03	4,69
Biotox - 3	43,29	43,34	0,15	1,47	4,60
Biotox - 4	50,11	51,13	0,13	1,04	5,17
Biotox - 5	42,50	42,85	0,14	1,57	4,94
Biotox - 6	49,50	49,14	0,17	1,66	4,02
Biotox - 7	27,50	29,14	0,08	1,08	8,21
Biotox - 8	30,63	30,73	0,13	1,13	5,20
Biotox - 9	45,90	46,15	0,14	0,91	5,06
Biotox - 10	42,77	42,23	0,15	0,94	4,66
Biotox - 11	49,72	49,27	0,15	1,16	4,59
Biotox - 12	40,78	40,33	0,14	1,20	4,88
Biotox - 13	44,25	43,86	0,14	1,09	5,00
Biotox - 14	31,06	30,69	0,18	0,97	3,74
Biotox - 15	Not enough sample				
Biotox - 16	43,71	43,37	0,18	0,5	3,78
Biotox - 17	Not enough sample				
Biotox - 18	41,01	40,91	0,16	1	4,35
Biotox - 19	35,22	35,06	0,18	0,93	3,83
Biotox - 20	39,77	40,24	0,14	0,93	4,78
Biotox - 21	41,58	41,37	0,18	1,1	3,90
Diesel	24,33	31,67	0,06	2	12,45

Table 15: Kinetic data calculated for each oils biodegradation

d) Conclusions

The first important information from the reported study is that all pyrolysis oils assessed are biodegradable as for each flask, an emission of CO_2 was measured. Bio-oil biodegradation starts immediately and no lag phases are observed at the beginning of a test. That indicates a very fast biological response (i.e., microbial growth, enzyme activity/synthesis). It means that some compounds in pyrolysis oils are immediately degraded by competent degraders from a conventional inoculum source: a sludge freshly collected from the aeration tank of a sewage treatment plant.

No lag phase at the beginning of tests (T_{lag}~1day) also reflects a rapid mass transfer of oil from the glass filters to the solution, which validates the method used to introduce oil samples in flasks. Biodegradation is also immediately observed at the beginning of the tests due to the acid nature of pyrolysis oils. In acidified aqueous solution, CO_2 is not dissolved and is directly removed as gas. This was verified by the evolution of the percentage of biodegradation at day 29, as the addition of 1ml acid solution in the medium engender a large emission of CO_2 only for the reference samples (which are not acid) and not for the oil samples.

All biodegradation curves have similar shapes and the values from replicates are close with an average deviation of degradability percentage between 0.5 and 2.4 %. Biodegradation curves show rapid degradation kinetics during the first 8 days (the growth phase) to reach a plateau (stationary phase) highlighting that pyrolysis oils biodegradation has virtually stopped between 22 to 26 days.

Based on the results presented, it appears that flash pyrolysis oils with biodegradation percentages between 32 and 50 % are slightly more biodegradable than gas oils (around 24 %) and have a much higher biodegradable potential than heavy fuel (11%).

I.2.2.5 Conclusions and selection of the representative sample for the full toxicological study

Assessment of the physico-chemical and the toxicology of the 21 collected samples gave a lot of information on Bio-oils.
These results reveal that despite being produced under differences conditions all the collected Bio-oils show similar properties, with minor variations in their physico-chemical composition and very similar toxicity (all mutagenic), ecotoxicity (no or light ecotoxicity) and same type of biodegradation (between 32 and 50 % and no lag phase)

Results of the characterisation of samples produced by Aston shown that

- The spruce oil is has a 3 fold higher viscosity than beech oil.
- The highest the pyrolysis temperature, the highest are the carbon content and the viscosity.
- Aging induce higher water content
- The highest the pyrolysis temperature, the highest is the PAH content.

A rigorous work was done to try to correlate oils productions parameters, to physico-chemical characteristics and to the toxicity, eco-toxicity and biodegradability. Three partners meetings were devoted to this work.

It clearly appeared that the number of potential influent parameters was too high, and variations of the results to low and despite all the collected data it was impossible to correlates all the results and in several cases just possible to elaborate hypothesis.

A goal of the engaged work was to investigate all the results of the tests done, in order to select the best parameters to produce a representative (standard) bio-oil sample.

No real bio-oil shows typical properties (except biotox 7 and 8), based on this statement the consortium selected the parameters to be used to produce the sample for the full toxicological and ecotoxicological tests:

- a temperature of 500°C : based on the results of the chemical analysis which allowed to determined the best pyrolysis temperature around 500°C to minimise the PAH level, it is also the temperature which maximize the liquid yield and will therefore be not appropriate for large scale production.
- spruce feedstock : soft wood is a typical European biomass
- fluidised bed process : this is the common industrial process used for bio-oil production

Three kilograms of this bio-oil were produced by Aston University. All the full toxicological and ecotoxicological tests were done by CIT.

I.2.3 Full toxicological study of one selected representative sample

One objective of the project was to notify bio-oils as a new substance to be placed on the EU market, following the actual notification way recommended nowadays within ELINCS (Eu List of Notified Chemical Substances).

For the notification a technical dossier has to be set up, containing data concerning physico-chemical; toxicological and ecotoxicological properties of the substance to be notified. Nature of these tests depends on the quantity of substance which is placed on the EU market. Procedures to be used are those set in Annex V of the Directive 92/32/CEE.

Based on the quantity of pyrolysis oils produced today in Europe, the steering comity selected to follow the BASET VII A of the Annex V of the Directive 92/32/CEE, valuable for quantity manufactured between 1 and 10 tonnes.

PHYSICO-CHEMICAL PROPERTIES	
A1	Freezing point (if > -20°C)
A1	Melting point
A2	Boiling point
A3	Relative density
A4	Vapour pressure - Static or gas saturation method
A5	Surface tension
A6	Water solubility - Flask or elution column method
A8	Partition coefficient n-octanol/water
A9	Flash point (liquids)
A10	Flammability (solids)
A12	Flammability : contact with water
A13	Autoflammability
A14	Explosive properties : 3 types
A15	Self-ignition temperature for a liquid
A16	Self-ignition temperature for a solid
A17	Oxidising properties (solids)
TOXICOLOGY STUDIES	
B1 tris	Acute oral toxicity (rat) - Acute toxic class method
B3	Acute dermal toxicity (rat) for a limit test at 2000 mg/kg
B4	Skin irritation (rabbit)
B5	Eye irritation (rabbit)
B6	Local lymph node assay : LLNA
B7	28-day oral toxicity study by gavage (rat)
7-day range-finding study for the 28-day study	
B14	Gene mutation in bacteria (Ames test)
B10	In-vitro chromosomal aberration in human lymphocyte
ECOTOXICOLOGY STUDIES	
C1	Acute toxicity to fish (trout) according to the WAF method
C2	Acute toxicity to daphnia according to the WAF method
C3	Algal growth inhibition according to the WAF method
C4	Biodegradability (modified Sturm test)
including activated sludge respiration inhibition test	
C7	Hydrolysis as a function of pH for a highly hydrolysable substance

Table 16: Notification of a new substance - base set - VII A
Commission Directive 92/32/EEC (Quantity > 1 tonne and < 10 tonnes)

Steering comity reviewed all the tests requirements from Annex VII A of the notification of a new substance. Based on the results of the screening tests and of CIT experience and due to their characteristics some tests are not valid for bio-oils. Based on this discussion, a selection of the tests to be done is presented in Table 17

PHYSICO-CHEMICAL PROPERTIES	
A6	Water solubility - Flask or elution column method
A8	Partition coefficient n-octanol/water
A14	Explosive properties : 3 types
TOXICOLOGY STUDIES	
B1 tris	Acute oral toxicity (rat) - Acute toxic class method
B3	Acute dermal toxicity (rat) for a limit test at 2000 mg/kg
B4	Skin irritation (rabbit)
B5	Eye irritation (rabbit)
B6	Local lymph node assay : LLNA
B7	28-day oral toxicity study by gavage (rat)
7-day range-finding study for the 28-day study	
B14	Gene mutation in bacteria (Ames test)
B10	In-vitro chromosomal aberration in human lymphocyte
ECOTOXICOLOGY STUDIES	
C2	Acute toxicity to daphnia according to the WAF method

Table 17: Selection of the feasible test to be carried out in the selected bio-oil sample

I.2.3.1 Tests A6 Water solubility, A8 Partition coefficient n-octanol/water and C2 Acute toxicity to daphnia

Tests A6 Water solubility, A8 Partition coefficient n-octanol/water and C2 Acute toxicity to daphnia require further chemical analysis to estimate the evolution of the test item concentrations in solution during the tests. But due to the nature of bio-oils, which are a mixture of more than 500 different compounds and not a purr substance, it is not possible to assess the evolution of it's concentration in solution.
Competent authorities (INERIS) were contacted in order to discuss the relevance of these tests for Bio-oils . It refused to give his opinion onto the necessity of leading the A8, A6 and C2 tests as REACH has not still officially started.
In dialogue with the experts of CIT we decided to stop these tests. These tests without chemical analyses to follow the evolution of the tested substances in the time are senseless.

I.2.3.2 -Test A14: Explosive properties

The objective of this study was to evaluate the potential of the test item Biotox-21 presents a danger of explosion when subjected to heat (heat sensitivity) or shock (mechanical sensitivity). Two types of tests were carried out:

Safety-in-handling tests:
The objective is to establish safe conditions for the performance of the assays of sensitivity in the main tests. Exposure of a very small sample (between 10 and 40 mm3) of bio-oil

- by heating, without any confinement, the sample directly with the flame of a gas burner,
- by subjecting the sample to impact (shock) in suitable apparatus,

The main tests concern a test of heat sensitivity by heating bio-oil in a stainless-steel tube with different degrees of confinement being provided by nozzle plates with holes of different diameters and a test of mechanical sensitivity (shock) by exposure of the bio-oil to the shock of a falling hammer on a steel anvil.

Heat sensitivity test (flame test)
Bio-oil was loaded into the tube to a height of 60 mm. The threaded collar was slipped onto the tube from below, the appropriate orifice plate was inserted and the nut tightened. The heating of tubes was provided by butane / propane, fitted with a pressure regulator, through a meter and evenly distributed by a manifold to four burners. The rate of gas is approximately 3.2 L/min. A test resulting in the fragmentation of the tube into three or more pieces, which in some cases may be connected to each other by narrow strips of metal, is evaluated as giving an explosion. A test resulting in fewer fragments or no fragmentation is regarded as not giving an explosion. Two series of three tests are mandatory: the first series using a nozzle plate with a hole of 6 mm diameter, the second using a hole of 2 mm diameter.

Six negative assays were recorded: no heat sensitivity was noted with the test item.

Mechanical sensitivity (shock)
The test item is considered as presenting a danger of explosion if:

- an explosion occurs (the tube bursts into three or more fragments) within the fixed number of tests for heat sensitivity or,
- an explosion (bursting into flame and/or a report equivalent to explosion) occurs at least once in six tests of mechanical sensitivity (shock).

For the main test a volume of bio-oil to reach 1 mm height in the cylinder was approximately 100 µL. Six negative assays were recorded. No shock sensitivity was noted with the bi-oil.

Conclusions

The Bio-oil sample is not considered to have explosive properties (heat and mechanical sensitivity) according to the experimental conditions.

I.2.3.3 -Test B4 : Dermal Irritation In Rabbit

The objective of this study was to evaluate the potential of the test item Biotox-21 to induce skin irritation following a single topical application to rabbits.
In the assessment of the toxic characteristics of a test item, determination of the irritant and/or corrosive effects on the skin of mammals is an important initial step. Information derived from this test serves to indicate the possible hazards likely to arise from exposure of the skin to the test item.
This study was conducted in compliance with:

- OECD guideline No. 404, 24th April 2002,
- Directive 2004/73/EC, B.4, 29th April 2004.

a) Methodology

The test item was applied undiluted on male New Zealand White rabbit. The day before treatment, both flanks of the animal were clipped using electric clippers and the skin of the animal was examined in order to check the absence of any signs of skin irritation. Bio-oil was first evaluated on a single animal. The durations of exposure were 3 minutes, 1 hour and 4 hours. Since the test item

showed corrosive properties on this first animal, the study was considered complete and the test item was not evaluated on other animals.

Doses of 0.5 mL of the undiluted test item were placed on a dry gauze pad, which was then applied to an area of approximately 6 cm² of the anterior left flank (application for 3 minutes), the anterior right flank (application for 1 hour) or the posterior right flank (application for 4 hours) of the animal. The untreated skin served as control.

After removal of the dressing, any residual test item was wiped off by means of a moistened cotton pad.

The skin was examined approximately 1 hour, 24, 48 and 72 hours after removal of the dressing. As severe irritant effects were observed, the animal was killed on day 4 (after the reading), for ethical reasons.

Dermal irritation was evaluated for each animal according to the following scoring scale:
Erythema and eschar formation:

- . no erythema.. 0
- . very slight erythema (barely perceptible) 1
- . well-defined erythema... 2
- . moderate to severe erythema.. 3
- . severe erythema (beet redness) to slight eschar formation (injuries in depth)...... 4

Oedema formation

- . no oedema 0
- . very slight oedema (barely perceptible)... 1
- . slight oedema (edges of area well-defined by definite raising) 2
- . moderate oedema (raised approximately 1 millimetre) 3
- . severe oedema (raised more than 1 millimetre and extending beyond area
- of exposure)... 4

Any other lesions were noted.

b) Interpretation of results and classification

The results obtained were evaluated in conjunction with the nature and the reversibility of the findings observed. Classification of the test item is based on the criteria laid down in Council Directive 67/548/EEC (on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.

Criteria for irritation: A substance or a preparation is considered to be irritating to the skin if, when it is applied to healthy intact animal skin for up to 4 hours, significant inflammation is caused and which persists for 24 hours or more after the end of the exposure period.

Criteria for corrosion :A substance or a preparation is considered to be corrosive if, when it is applied to healthy intact animal skin, it produces full thickness destruction of skin tissue on at least one animal during the test for skin irritation, or if the result can be predicted (for example: from strongly acid or alkaline reactions).

Classification:
Irritant substances

- symbol Xi, indication of danger "irritant",

- phrases indicating the nature of special risks: R 38: "Irritating to skin" Inflammation is significant if: the mean value of the scores is two or more for either erythema and eschar formation or oedema formation. The same will be the case where the test has been completed using three animals if the score for either erythema and eschar formation or oedema formation observed in two or more animals is equivalent to the value of two or more, it persists in at least two animals at the end of the observation period. Specific effects such as hyperplasia, desquamation, discolouration, fissures, eschar and alopecia should be taken into account.

Corrosive substances

- symbol C, indication of danger: "corrosive",
- phrases indicating the nature of special risks:.
    - R 34: "Causes burns" If, when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of up to 4 hours exposure, or if this result can be predicted..
    - **R 35:** "Causes severe burns"If, when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of up to 3 minutes exposure, or if this result can be predicted.

c) Results

After a 3-minute exposure:

A very slight erythema (grade 1) was observed on days 2 and 3.
No other cutaneous reactions were noted.
A beige coloration of the skin, due to the test item, was noted from day 1; it persisted up to the end of the observation period (day 4).
Mean scores over 24, 48 and 72 hours were 0.7 for erythema and 0.0 for oedema.

After a 1-hour exposure:

A well-defined erythema (grade 2; days 1, 2 and 3) then a severe erythema (grade 4; day 4) associated with a brownish area similar to a severe burn of the skin, were noted.
A slight oedema (grade 2) was noted from day 2 up to day 4.
A beige coloration of the skin, due to the test item, was noted from day 1 up to day 3.
Mean scores over 24, 48 and 72 hours were 2.7 for erythema and 2.0 for oedema.

After a 4-hour exposure:

A well-defined erythema (grade 2; days 1, 2 and 3) then a marked erythema (grade 3; day 4) were observed.
A very slight oedema (grade 1) was observed on days 3 and 4.
A dryness of the skin was recorded on day 4.
A beige coloration of the skin, due to the test item, was noted from day 1 up to day 4.
Mean scores over 24, 48 and 72 hours were 2.3 for erythema and 0.7 for oedema.

d) Conclusions

Under the experimental conditions, the test Bo-oil Biotox-21 is corrosive when applied topically to rabbits. According to the classification criteria laid down in Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labeling of dangerous substances, the bio-oil Biotox-21 should be classified as corrosive and assigned the symbol C, the indication of danger "Corrosive" and the risk phrase R 34: "Causes burns".

I.2.3.4 –Test B3: Acute Dermal Toxicity in Rat and B5 Eye Irritation in Rabbit

As the "B4 Dermal Irritation In Rabbit" test shown that bio-oils are corrosives, tests **B3 Acute Dermal Toxicity In Rat** and **B5 Eye Irritation In Rabbit** were cancelled for ethic reasons as recommended by the Commission Directive 92/32/EEC.

I.2.3.5 –Test B1 tris: Acute Oral Toxicity in Rat

The objective of this study was to evaluate the toxicity of the test item BIOTOX-21 following a single oral administration in rats. In the assessment of the toxic characteristics of a test item, determination of acute oral toxicity is an initial step. It provides information on health hazards likely to arise following a short-term exposure by the oral route in humans and enables the test item to be ranked in different classification systems.

The acute toxic class method is a stepwise procedure. The test item is administered orally to one group of animals at one of the defined dose-levels, each step using three females. Absence or presence of compound-related mortality of the animals dosed at one step determines the next step, i.e.:

- no further testing is needed,
- the next step is performed with the same dose,
- the next step is performed at the next higher or the next lower dose-level.

The study was conducted in compliance with:

- EC Directive 2004/73/EC, B.1 tris, 29th April 2004,
- OECD Guideline No. 423, 17th December 2001.

a) Methodology

Bio-oil was prepared at the chosen concentration in the vehicle (propylene glycol).
The animals were fasted for an overnight period of approximately 18 hours before dosing, but had free access to water. Food was given back approximately 4 hours after administration of the test item. Three females were used for each step. The dose-level used as the starting dose was selected from one of four fixed levels, 5, 50, 300 or 2000 mg/kg body weight. As the information on the toxic potential of the test item suggested that mortality was unlikely at the highest dose-level, a limit test was performed, using the starting dose-level of 2000 mg/kg with three animals. As no deaths occurred, the results were then confirmed in three other females.
The study design was as follows:

Dose(mg/kg)	Volume(mL/kg)	Female
2000	10	3
2000	10	3

The dosage form preparations were administered to the animals under a volume of 10 mL/kg. The administration was performed in a single dose by oral route using a metal gavage tube fitted to a 2 mL plastic syringe (0.1 mL graduations). The volume administered to each animal was adjusted according to body weight determined on the day of treatment. The single administration was performed in the morning of day 1; it was followed by a 14-day observation period.

The animals were observed frequently during the hours following administration of the test item, for detection of possible treatment-related clinical signs. The animals were weighed individually just before administration of the test item on day 1 and then on days 8 and 15. The body weight gain of the treated animals was compared to that of CIT control animals with the same initial body weight.

b) Results

No mortality was noted during the study.

Hypoactivity or sedation, piloerection and dyspnea (all animals); unsteady gait (3/6 animals), were observed within 6 hours of treatment.

No more clinical signs persisted on day 2, up to the end of the study.

The overall body weight gain of the treated animals was similar to that of CIT historical control animals. Macroscopic examination of the main organs of the animals revealed no apparent abnormalities.

c) Conclusions

Under the experimental conditions, the oral LD_{50} of the test item BIOTOX-21 is higher than 2000 mg/kg in rats.

I.2.3.6 –Test B6 : Evaluation of skin sensitization potential in Mice Using Local Lymph Node Assay (LLNA)•

The aim of this study was to evaluate the potential of the test item BIOTOX-21 to induce delayed contact hypersensitivity, using the murine Local Lymph Node Assay (LLNA

The study has been designed to comply with the following guidelines:
. OECD Guideline No. 429, 24th April 2002,
. EC Directive No. 2004/73/EC, part B.42, 29th April 2004.

a) Methodology

A preliminary test was first performed in order to define the concentrations of test item to be used in the main test.

In the main test, twenty-eight female CBA/J mice were allocated to seven groups:

- five treated groups of four animals receiving the test item BIOTOX-21 at the concentration of 0.5, 1, 2.5, 5 or 10%,
- one negative control group of four animals receiving the vehicle (dimethylformamide =DMF),
- one positive control group of four animals receiving the reference item, α-hexylcinnamaldehyde (HCA), a moderate sensitizer, at the concentration of 25%.

During the induction phase, bio-oil, vehicle or reference item was applied over the ears (25 µL per ear) for 3 consecutive days (days 1, 2 and 3). After 2 days of resting, the proliferation of lymphocytes in the lymph node draining the application site was measured by incorporation of

tritiated methyl thymidine (day 6). The obtained values were used to calculate stimulation indices (SI).
The irritant potential of the test item was assessed in parallel by measurement of ear thickness on days 1, 2, 3 and 6.

b) Results

The administration of the undiluted test item was not possible. The undiluted form of the test item could not pass through a micropipette.
Due to the unsatisfactory solubility of the test item in the first recommended vehicle (acetone/olive oil (4/1, v/v)), DMF was chosen among the other proposed vehicles.

A homogeneous dosage form preparation was obtained at the maximal concentration of 50%. Consequently, the concentrations selected for the preliminary test were 5, 10, 25 and 50%.

Since the test item was considered as irritant in the preliminary test at the concentrations of 25 and 50%, the highest tested concentration retained for the main test was 10%.

Systemic clinical signs and mortality: No mortality and no clinical signs were observed during the study.

Local irritation: No cutaneous reactions and no noteworthy increase of ear thickness were observed in the animals of the treated groups.

Proliferation assay: A significant lymphoproliferation (SI > 3) was noted at the tested concentrations of 5 and 10% (SI = 6.35 and 14.58, respectively), as well as in the positive control group given HCA (SI = 3.99).
The results are presented in table Table 18.

Treatment	Concentration (%)	Signs of local irritation	Stimulation Index (SI)
Test item	0.5	no	1.19
Test item	1	no	2.48
Test item	2.5	no	1.73
Test item	5	no	6.35
Test item	10	no	14.58
HCA	25	-	3.99

Table 18: Results of the "*Evaluation of skin sensitization potential in Mice Using LLNA*" test•

In the absence of local irritation, the observed lymphoproliferative responses were attributed to delayed contact hypersensitivity. The EC_3 value for the test item BIOTOX-21 is equal to 3.19%.

c) Conclusions

Under the experimental conditions, the test item BIOTOX-21 induces delayed contact hypersensitivity in the murine Local Lymph Node Assay.

According to the EC_3 value obtained in this experiment and to the categorization of contact allergens by Kimber I. and al. (2003), the test item BIOTOX-21 should be considered as a moderate sensitizer.

I.2.3.7 –Test B7: 7-day study oral route in rats

The objective of this study was to evaluate the potential toxicity of the test item, BIOTOX-21, following daily oral administration (gavage) to rats for 7 days.

The rat was chosen because it is a rodent species commonly accepted by regulatory authorities for this type of study. The oral route was selected since it is a route of exposure which is requested by the authorities. The dose-levels were selected in agreement with the Sponsor on the basis of the results of an acute toxicity study by oral route performed in the same species *(Test B1 tris: Acute Oral Toxicity in Rat).*

This study was based on the following guidelines:

. OECD Guideline No. 407, 27th July 1995,
. EEC Directive No. 96/54, B7, 30th September 1996.

a) Methodology

Three groups of three male and three female Sprague-Dawley rats received the test item, BIOTOX-21, daily, by oral (gavage) administration, for 7 days, at dose-levels of 150, 500 or 1500 mg/kg/day. Another group of three males and three females received the vehicle, propylene glycol, under the same experimental conditions and acted as a control group. The dosing volume was 5 mL/kg.

Clinical signs and mortality were checked daily. Body weight was recorded three times and food consumption twice during the dosing period. On completion of the dosing period, the animals were sacrificed and a complete macroscopic examination was performed. Selected organs were weighed and any macroscopic lesions were preserved.

b) Clinical examinations

Morbidity and mortality: Each animal was checked for mortality or signs of morbidity at least twice a day during the treatment period, including weekends and public holidays, and at least once a day during the acclimation period.

Clinical signs: Each animal was observed at least once a day, at approximately the same time, for the recording of clinical signs.

Body weight: The body weight of each animal was recorded at least once before group allocation, on the first day of treatment, and on days 4 and 7.

Food consumption: The quantity of food consumed by each animal was recorded twice, over 3- or 4-day periods, during the treatment period.

c) Pathology

Sacrifice: On completion of the treatment period, after at least 14 hours fasting, all animals were sacrificed by carbon dioxide inhalation and exsanguination.

Organ weights: The body weights of all animals sacrificed at the end of the treatment period were recorded before sacrifice. The adrenals, brain, heart, testes and epididymides, kidneys, liver, lungs, ovaries, spleen and thymus were weighed wet as soon as possible after dissection. The ratio of organ weight to body weight (recorded immediately before sacrifice) was calculated.

Macroscopic *post-mortem* examination: A complete macroscopic *post-mortem* examination was performed on all study animals. This included examination of the external surfaces, all orifices, the cranial cavity, the external surfaces of the brain, the thoracic, abdominal and pelvic cavities with their associated organs and tissues and the neck with its associated organs and tissues.

Preservation of tissues: For all study animals, macroscopic lesions were preserved in 10% buffered formalin.

d) Statistical analysis

The specific sequence was used for the statistical analyses of body weight, food consumption, and organ weight data.

e) Results

There were no premature deaths during the study.

All animals given 1500 mg/kg/day had hypersalivation throughout the treatment period and one female also had loud breathing and piloerection. No clinical signs were observed at 500 or 150 mg/kg/day.

There was a dosage-related reduction in mean body weight gain for females given 150, 500 or 1500 mg/kg/day and for males given 500 or 1500 mg/kg/day between day 1 and day 4 of dosing. From day 4 of dosing all groups had body weight gains comparable with the controls except the males given 1500 mg/kg/day who continued to have reduced body weight gains and statistically significantly reduced body weights.

Males given 1500 mg/kg/day had reduced food consumption throughout the study while females given 1500 mg/kg/day and males given 500 mg/kg/day had reduced food consumption just for the first half of the dosing period. There was no effect at 150 mg/kg/day or for females given 500 mg/kg/day.

At necropsy, all animals given 1500 mg/kg/day had yellowish-colored, thickened forestomachs and most had dilated duodenums. One male given 500 mg/kg/day had a dilated duodenum.

The mean absolute and relative spleen weights were reduced for both males and females at 1500 mg/kg/day. The mean absolute and relative liver and ovary weights were increased for females given 1500 mg/kg/day. The mean absolute and relative thymus weights were reduced for males given 1500 mg/kg/day.

f) Conclusions

Hypersalivation was observed for all animals at 1500 mg/kg/day with at least an initial reduction in food consumption and body weight gain and, for the males, statistically significantly reduced body weights. At necropsy, yellowish-colored, thickened forestomach were observed with dilated duodenums. Spleen weights were reduced for males and females, thymus weights were reduced for the males and liver and ovary weights were increased for the females.

At 500 mg/kg/day, there were no clinical signs, but there was a reduction in body weight gain and, for males, a reduction in food consumption. One male had dilatation of the duodenum.

At 150 mg/kg/day, there were no clinical signs of toxicity, a slight reduction in the body weight gain of the females and no effect on food consumption.

No macroscopic abnormalities were observed.

I.2.3.8 –Test MAS In Vivo: Bone marrow micronucleus test by oral route gavage in mice

The objective of this study is to evaluate the potential of the test item to induce damage to the chromosomes or the mitotic apparatus in bone marrow cells of mice. Apart from detecting chromosome breakage events (clastogenesis), the micronucleus test is capable of detecting chemicals which induce whole chromosome loss (aneuploidy) in the absence of clastogenic activity.

In the bone marrow of mice exposed to a chemical which induces cytogenetic damage, chromosomal fragments or entire chromosomes which are left behind at cell division will not be incorporated into the nuclei of daughter cells. Most of these fragments condense and form one or more micronuclei in the cytoplasm. The visualization of micronuclei is facilitated in erythrocytes because their nucleus is extruded during erythropoiesis. Accordingly, the basis of this test is an evaluation of the increase in the number of micronucleated polychromatic erythrocytes (MPE). Substances which inhibit either proliferation or maturation of erythroblasts and those which are toxic for nucleated cells, decrease the proportion of immature erythrocytes (polychromatic, PE) when compared to mature erythrocytes (normochromatic, NE). Thus, the cytotoxicity of a substance can be evaluated by a decrease in the PE/NE ratio.

This study has been designed to comply with the following guidelines:

- OECD guideline No. 474, adopted on 21st July 1997.
- Commission Directive No. 2000/32/EC, B12, 8 June 2000.

a) Methodology

Three dose-levels, administered orally twice separated by 24 hours, were used with one single sampling time (see Table 19) .

Treatment	Animals per group		Target dose-level mg/kg/day	Sampling time after the last treatment
Vehicle	Principal	5 males 5 females	0	24 h
Low dose-level	Principal	5 males 5 females	(1)	24 h
Intermediate dose-level	Principal	5 males 5 females	(1)	24 h
High dose-level	Principal	8 males (2) 8 females (2)	(1)	24 h
	Satellite	3 males (3) 3 females (3)		
Positive control CPA	Principal	5 males 5 females	50	24 h

(1) Will be determined on the basis of the results of the preliminary test
(2) Only the five surviving animals of each sex will be subjected to bone marrow analysis.
(3) Satellite animals allocated for determination of plasma level of the test item (see § 4.2.3).

Table 19: Methodology applied for the" Bone marrow micronucleus test by oral route gavage in mice" test

Blood samples for the determination of plasma levels of the test item were taken following the second treatment.

At the time of sacrifice, all the animals were killed by CO_2 inhalation in excess. The femurs were removed and the bone marrow were flushed out with fetal calf serum. After centrifugation, the supernatant were removed and the cells in the sediment were resuspended by shaking. A drop of this cell suspension was placed and spread on a slide. The slides were coded so that the scorer is unaware of the treatment group of the slide under evaluation ("blind" scoring).

For each animal, the number of micronucleated polychromatic erythrocytes (MPE) were counted, the polychromatic (PE) and normochromatic (NE) erythrocyte ratio was established by scoring a total of 1000 erythrocytes (PE + NE).

The analysis of the slides was performed at CIT or at Microptic, cytogenetic services (2 Langland Close Mumbles, Swansea SA3 4LY, UK).

b) Results

No significant increase in the frequency of micronucleated cells was observed in animals treated with the bio-oil sample. Analyses on blood samples would be necessary to confirm inactivity of the bio-oil sample.

I.2.3.9 – Test MNV In Vitro: Micronucleus test in L5178 TK+/- mouse lymphoma cells

The objective of this study is to evaluate the potential of the test item to induce any increase in the frequency of micronucleated cells. The micronuclei observed in the cytoplasm of interphase cells may originate from acentric fragments (chromosome fragments lacking a centromere) or whole chromosomes that are unable to migrate with the rest of the chromosomes during the anaphase of cell division. The assay thus has the potential to detect the activity of both clastogenic and

aneugenic chemicals. This test will be performed in the absence and presence of a rat liver metabolising system (S9 mix).

This study has been designed to comply with the following guidelines:
· OECD guideline No. 487, draft dated June 14, 2004.
Preliminary toxicity test :

a) Methodology

To assess the cytotoxicity of the bio-oil sample, six dose-levels (one culture/dose-level) were tested both with and without metabolic activation. Assessment of cytotoxicity was performed by evaluation of population doubling (PD) (e).
The population doubling is the log of the ratio of the final count at the time of harvesting (N) to the starting count (N0), divided by the log of 2.
PD = [log (N/N0)]/log 2.

Mutagenicity experiments :
In two independent experiments, four dose-levels of bio-oils (two cultures/doselevel) were tested both with and without metabolic activation, using treatment duration as follows:

	First experiment	Second experiment
Without S9 mix	3 h treatment + 24 h recovery	24 h treatment + 0h recovery*
With S9 mix	3 h treatment + 24 h recovery	3 h treatment + 24 h recovery

** if the results of the first experiment are negative or equivocal*

Each treatment was performed in duplicate as follows:

For treatment, approximately 3 x 10^5 cells/mL (final concentration = N0) in RPMI 1640 medium containing 5% inactivated horse serum with or without 5% S9 mix, were exposed to the bio-oil or control item.

At the end of the treatment period which will be performed at 37°C in a humidified atmosphere of 5% CO_2/95% air, the cells were washed twice. Cells were suspended in RPMI 1640 medium containing 5% inactivated horse serum and the plates were incubated, at 37°C in a humidified atmosphere of 5% CO_2/95% air, for the recovery period, if any.

At the end of the recovery period, if any, the cells were washed with RPMI 1640 medium containing 10% inactivated horse serum containing 1% pluronic acid. The cells were suspended in a medium with 49.5% RPMI 1640 medium containing 10% inactivated horse serum, 50% PBS and 0.5% pluronic acid, before being fixed.

Depending on the observation at the end of the recovery period (presence or absence of precipitate and/or cytotoxicity), at least three dose-levels of bio-oil treated cultures were selected for spreading of slides in the view of slide analysis. Cells were dropped onto clean glass slides. Each treatment wascoupled to an assessment of cytotoxicity at the same dose-levels.

Cytotoxicity was evaluated by determining the PD of cells and was also take into account the quality of the cells on the slides.
The slides were coded before analysis performed under a microscope (1000 x magnification).

b) Results

Without S9 mix:

In the second experiment (24-hour treatment), a dose-related tendency is observed in the frequency of micronucleated cells with a significant difference at 15.63 µg/mL. These results come in addition to the significant result (although not dose-related) noted in the first experiment (3-hours treatment).It is necessary to perform confirmatory assays, ideally for the two experimental conditions (3-hour and 24-h treatments) in order to judge of the relevance of the observed results

With S9 mix:

In the second experiment, a dose-related tendency is observed in the frequency of micronucleated cells with a significant difference at 75 µg/mL. The equivalent of this dose-level of 75 µg/mL could not be analyzed in the first experiment, and therefore it is difficult to judge of the reproducibility of this effect, all the more since only at this dose that the decrease of doubling population reachs the 50% level. It would be consequently necessary to perform a confirmatory experiment, using a closer range of dose-levels.

c) Conlusion

Under the experimental conditions, the test item Biotox-21 presented a light mutagenic activity, which has to be confirm through further experiment

I.2.3.10 – Conclusions of the full toxicological characterisation of the selected sample

All the planned tests were carried out without problems, it appears that bio-oil is:

- not explosive when subjected to heat or shock *(A14 test),*
- should be classified as “corrosive” and assigned the symbol C, the indication of danger “Corrosive” and risk phrase R34: “ Causes Burns” *(B4 test),*
- “Moderate sensitizer” and assigned the risk phrase R 43: “May cause sensitization by skin contact“ *(B6 test),*
- not toxic by oral route *(B1 tris test)* and by 7-days oral gavage *(B7 test),*
- not mutagenic via *In Vivo MAS* test but shown a light mutagenic effect via *In Vitro MNV test*.

Studies *B3 Acute Dermal Toxicity In Rat* & *B5 Eye Irritation In Rabbit* were cancelled, for ethic reason, due to the corrosive behaviour of bio-oils substance

I.2.4 Recommendations for safety procedures and dissemination of the results

Main objective of Biotox is to ensure that all obtained results within the project will benefit to the flash pyrolysis oil community. Three main works were planned: dissemination of the results, editions of safety documents based on the obtained results, and notification of Bio-oil as a new substance to be placed on the Eu market.

I.2.4.1 Dissemination

In a first time bio-oils producers were informed and consulted (to choose samples to be collected and relevant analyses to be carried out) concerning the projects trough experts meetings and conferences (see chapter management). They were invited to take part to the project by producing oils sample for the projects.

All the results were presented several times through several conferences and publications:

a) Conferences and experts meetings

- Conference: *Pyrolysis and gasification of biomass and waste,* in Strasbourg, 30 September-1 October 2002, Poster presentation and proceeding *Pyrolysis and Gasification of Biomass and Waste, A. V. Bridgwater*, CPL press, p155;

- PyNe Expert meeting in Florence, 2-6 April 2003. Oral presentation and proceeding;

- PyNe Expert meeting in Bruges, 15-18 April 2004. Oral presentation and proceeding;

- Conference: Science *in Thermal and chemical Biomass Conversion Conference in Victoria* (30 august to 2 september 2004). Poster presentation.
  Proceeding: J. Blin, G. Volle, N. Maghnaoui & P. Girard; "Biodegradability of fast pyrolysis" Science in thermal and chemical biomass conversion – Vancouver Island – Canada –Sept. 2004, *A. V. Bridgwater*, CPL press, in press;

- Conference: Science *in Thermal and chemical Biomass Conversion Conference in Victoria* (30 august to 2 September 2004). Oral presentation.
  Proceeding: Girard P. Blin J. "Bio-oil toxicity for safe handling and transportation " Science in thermal and chemical biomass conversion – Vancouver Island – Canada –Sept. 2004; *A. V. Bridgwater*, CPL press, in press;

- PyNe Expert meeting in Innsbrück, 27-30 September 2005. Oral presentation;

- Results of the project as been selected for an oral presentation in the international conference: "The 14th European Conference and Technology Exhibition on Biomass for Energy, Industry and Climate Protection" scheduled to take place from the 17th to 21st October 2005 in Paris.

b) Published articles

- P. Girard & J. Blin. "Environment, Health and Safety Aspects Related to Pyrolysis oils". **2005**; PyNe Handbook Volume 3, chap 4, P 61-70, A. V. Bridgwater, CPL press, UK

- J. Blin, P. Girard, G. Volle. "Biodegradability of Fast Pyrolysis Oil". **2005**, PyNe Handbook Volume 3, chap 5, P 71-94, A. V. Bridgwater, CPL press, UK.

- J. Blin, P. Girard, G. Volle."Bio-oil toxicity assessment versus pyrolysis parameters". **2005**, PyNe Handbook Volume 3, chap 6, P95-104, A. V. Bridgwater, CPL press, UK.

- A complete paper presenting Biodegradability work carried out within Biotox has been submited to be published in the international academic journal Chemosphere*: Aerobic Biodegradability of Biomass Pyrolysis Oils*; Joël Blin, Ghislaine Volle, Philippe Girard, Anthony Bridgwater, Dietrich Meier, Draft for *Chemosphere.*

Biotox was also introduced in March 2003 in the Pyne newletter: Paper in issue N° 15 available to download in PDF format http://www.pyne.co.uk/docs/PyNews%2015.pdf ;

A book containing all the results of the project, with details on all analytical methods is under preparation to be published in the form of a "fast pyrolysis handbook n°4.

c) Web site:

A web pages hosted by the PyNe site is dedicated to Biotox http://www.pyne.co.uk/?_id=29.
This web page will be used in following months to disseminate all the publishable results of the project (publishable report, report on transport requirements and guidelines, MSDS, ..), as accepted by the commission.

I.2.4.2 Notification of Bio-oil as a new substance to be placed on the Eu market

According to EU legislation since 1981, news substances introduced in the internal market must undergo an in-depth risk assessment to examine the risks posed to humans and the environment New subsatnces are then notified in **ELINCS** (Eu List of Notified Chemical Substances 4000 entries). Risk assessment follows the framework set out in Regulation 1488/94 and Directive 93/67. Before, substances introduced between 1971 and 1981 were listed in the **EINECS** (EU Inventory of Existing Commercial Chemical Substances 100 196 entries).

The present system for general industrial chemicals distinguishes between "existing substances in **EINECS** " i.e. all chemicals declared to be on the market in September 1981, and "new substances in **ELINCS** " i.e. those placed on the market since that date.

At the beginning of the project, members of the flash pyrolysis thought that Bi-oils were not registred as an existing substances witch can be transported and manufactured within the EU market. Several bibliographic work and consultations of competent Eu authorities confirmed this observation. To reply on oils manufacturers' request, steering comity decided within Biotox to set up the technical dossier to be submitted to the European Competent Authorities to notify bio-oil as a new chemical substance. This choice influenced the selection of the tests carried out for the full toxicological characterisation of the representative selected oil (see chapter II.2.4).

But during the first year of the project, investigations on the data list of the European Chemicals Bureau (ECB) allowed to find that products obtained by pyrolysis of wood were registered in the EINECS data list, which definition fit well with fast pyrolysis bio-oil.

- Name:*Wood, hydropyrolysed*
- Definition: *A complex combination of organic compounds obtained from the thermal decomposition of wood*
- Cas n° : *94114-43-9*
- Einecs n° : *302-678-6*

Examination of the EINECS notification dossier, reveal that pyrolysis oils were only registered with a name, a full definition, an EINECS number and CAS number. However, neither other data (characterisations, requirement and guidelines for transport and storage) nor MSDS are available.

Competent authorities (European Chemicals Bureau and INERIS) were contacted in order to know if it is necessary to notify Bio-oils as a new substance in ELINCS as it is registered in the existing substances list EINECS. They confirmed that because pyrolysis oil is described in the EINECS data list with EINECS and CAS numbers, it is not mandatory to do a new notification. These two numbers are the only required data for legal commercialisation of products in Europe today.

In addition, since the project started, the legislation for production, transport and storages of substances within the Eu market has been planned to change in the coming years.

Today, there are only 2,700 new substances (in ELINCS). Testing and assessing their risks to human health and the environment according to Directive 67/548 are required before marketing in volumes above 10 kg. Indeed, following EU legislation, Directive 93/67/EEC and Commission Regulation (EC) N°. 1488/94, the testing requirements are tiered according to the volume placed on the market. The lowest volume triggering the need for testing amounts to 10 kg. More extensive testing is required when the volume reaches 100 kg, 1 t, 10 t, 100 t and 1,000 t, respectively. Generally, testing requirements at the lower volumes (10 kg to 1 t) focus on acute hazards, immediate or slightly delayed effects after short term exposure, while those at the higher tonnage include more expensive studies on the effects of sub- chronic exposure, on reproductive toxicity and on carcinogenicity. The testing package at 1 t is termed 'base set' while those triggered by higher tonnage are called Level 1 (100 t) and Level 2 (1,000 t).

Existing substances (in EINECS) amounting for more than 99% of the total volume of all substances on the market are not subject to the same testing requirements. The number of existing substances reported in 1981 was 100 106, the current number of existing substances marketed in volumes above 1 tonne is estimated at 30 000. Some 140 of these substances have been identified as priority substances and are subject to comprehensive risk assessment carried out by Member State authorities.

In contrast to new substances, existing substances have never been subjected to systematic testing regime. When the requirement for testing and notification of new substances was introduced in 1981, substances already on the market were exempted.

The gap in knowledge about intrinsic properties for existing substances should be closed to ensure that equivalent information to that on new substances is available. Therefore, the Commission proposes that existing and new substances should in the future, following the phasing in of existing substances until 2012, be subject to the same procedure under a single system. In October 2003 the European Commission adopted a legislative proposal for implementing a new EU chemicals policy. The new regulatory system, known as REACH (Registration, Evaluation and Authorization of Chemicals), comprises four procedures.

1. *Registration* of chemicals, documenting that risk is adequately controlled. Manufacturers and importers of chemicals in the EU must register those substances they produce or market in quantities above 1 tonne per year (tpa). Phase-in periods are proposed for some 30000 existing substances, with substances manufactured or imported in tonnage above 1000 tpa being registered first, followed by 100 tpa and 1tpa. In order to avoid multiple testing of the same substance,

REACH aims to encourage industries to form consortia and gather information into a central database.

2. *Evaluation* of the registration dossiers, considering mainly testing proposals. Member-state authorities will evaluate testing proposals for all substances manufactured or imported in quantities above 100 tpa (dossier evaluation) and any other prioritized substances if deemed necessary (known as a substance evaluation).

3. *Authorization* of substances of very high concern. These include substances that are carcinogenic, mutagenic or toxic to reproduction and substances with a potential for persistence and bioaccumulation combined with high (eco-)toxicity or very persistent and very bio accumulative substances.

4. *Restriction* of substances at the level of the European Community when risk reduction measures proposed by industry are not sufficient. Restrictions can be considered as a "safety net" allowing EU member states and/or the Commission to address risks that are not managed adequately by other parts of the REACH system.

REACH will implement the objectives set out in the "White paper: Strategy for a Future Chemicals Policy"].It will give industry the responsibility for ensuring and demonstrating the safe manufacture, use and disposal of chemicals. This represents a shift of responsibility to demonstrate and manage risk(s) associated with the use of chemicals from authorities to the actors in the chemical supply chain. As a result, manufacturers and importers of chemicals are required not only to control risks present during those stages of a substance's life cycle under their direct control but also to give guidance for use by downstream users on the safe handling and use of the substance.

So with REACH, it is planned that EINECS registration will be examined to identify the missing data for safe handling, transportation and used. However, because REACH is under discussion and will probably not be operational before 2006, it is not possible to enrich the existing EINECS data base.

The competent authority suggested us to compile all collected data to set up a full dossier base on ELINCS requirement, the SNIF summary (SUMMARY NOTIFICATION INFORMATION FORMAT) , with MSDS in order to prepare REACH.

I.2.4.3 Editions of safety documents based on the obtained results

Based on the obtained results from the physico-chemical and screening toxicological tests of the 21 oils Care set up by a full report requirements for "transport, storage and handling of biomass derived fast pyrolysis liquids; compliance with all international modes of transport" (free available on the Pyne Web site).

This report addresses the legislative requirements and regulations for the safe transport and labelling of pyrolysis liquids.

An article on "Environment, Health and Saftey Aspects Related to Pyrolysis oils" was published in PyNe Handbook Volume 3, (P. Girard & J. Blin. 2005; chap 4, P 61-70, A. V. Bridgwater, CPL

press, UK). This paper review the Risks involved in bio-oil production and use, and the environmental risk assessment in the Eu context.

An other article intituled “Biodegradability of Fast Pyrolysis Oil” was published PyNe Handbook Volume 3 (J. Blin, P. Girard, G. Volle, 2005, chap 5, P 71-94, A. V. Bridgwater, CPL press). In this report the methodology to be applied to assess bio-oils biodegradability is described as well as the results obtained within Biotox.

A complete MSDS with all data collected within the project is available on the Pyne Web site.

All these documents on secure handling of bio-oils will be registered on PyNe web site with free access and download when the commission will have validated the final report.

I.3. Acknowledgements

Biotox is a RTD project funded through the European Commission's Energy, environment and sustainable development Programme under Framework Programme. The Biotox Partnership is very grateful to the European Union for providing financial support (under contract number NNE5-2001-00744). The Biotox Partnership thanks the project officer from the Commission, Mr. José Riesgo Villanueva, for his support over the period of the grant.