EXPERT REPORT

Ecotoxicological evaluation on the application of “Bioversal” in combating mineral oil contaminations in soil and water

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1. Emission and ecological relevance of mineral oil contaminations

At a global emission of 88 million tonnes per year (PARLAR u. ANGERHÖFER 1991) ecosystem contamination with hydrocarbons from mineral oil and mineral oil products represents a worldwide problem, intensely affecting marine ecosystems in particular. 35% of these global emissions impact continental/terrestrial and aquatic (freshwater) ecosystems (ALBERS 1995), whereby particularly high contaminations are to be expected under certain conditions and in locally restricted areas. This also applies to Austria and its neighbouring European countries. Contamination sources coming into question here are motor vehicle traffic in the broadest sense (including its necessary infrastructure), shipping traffic, air traffic, continental oil drilling systems, refineries and their infrastructure, waste incineration systems and waste disposal sites, as well as all other consumers of fossil energy sources. A particular problem is massive contaminations with larger amounts of mineral oil or mineral oil products (crude oil, petrol, diesel oil, fuel oil, etc.), which primarily enter soil and water undiluted due to accidents or unintentional releases.

The danger to the environment stemming from such contaminations is considerable and varied. Mineral oil products, as a result of their hydrophobia, tend to form closed single-phase streaks or highly viscous water-in-oil emulsions on the water surface. Some particularly heavy refinery products sink below the surface of the water and cover sediments and surfaces of organisms with a silty coat or a thin film. Particularly sensitive are aquatic littorals, in which mineral oil coats the ecologically often particularly valuable littorals between water and the surrounding area and can cause lasting contamination. Furthermore, hydrocarbons released from mineral oils tend to be absorbed in the surfaces of sediments and aquatic organisms, due to their chemical/physical characteristics, thereby reducing their mobility and availability.

In terrestrial ecosystem contamination the high capillarity, thus the high penetrability of mineral oil products in the soil's capillary zones, plays an important role. In the soil itself hydrocarbons from mineral oil products exhibit a high absorption capacity, which increases as a rule with the content of organic substance. The half-life period of the retention of mineral oil products in soils can amount to decades without human assistance, and aromatic hydrocarbons can still be demonstrated in mineral oil-contaminated soil up to 20 years after the contamination event (ALBERS 1995). Following contamination, an aging process as a rule begins in the soil, in the course of which a part of the hydrocarbons contained in the mineral oil can initially be degraded. As a rule degradation gradually comes to a standstill, and the availability of the remaining hydrocarbon compounds drops dramatically through the absorptions process. This type of contaminated and aged soil is nearly impossible to clean and represents an uncomfortable and dangerous contaminated site.

The first scientific investigations on the ecological effects of crude oil contaminations were published by SMITH (1970) on the occasion of the “Torrey Canyon” tanker catastrophe off the British southwestern coast in March 1967. Since then, the prevailing realisation has been that more or less closed crude oil slicks on the high seas represent little danger to the environment as crude oil under such conditions only has a slightly toxic effect on organisms and furthermore loses most of its volume within a brief period, thanks to the volatility of low molecular components, photolytic disintegration and biological degradation. As its specific weight rises above that of the water the remaining oil agglutinates and sinks to the soil, where it is subjected to further, slow biological degradation. The site where closed mineral oil spills or oil slicks reach beaches and littorals proves more difficult. In such areas as a rule there is a far greater variety of species than on the high seas and the risk of physical harm to organisms through silting and toxic effects of hydrocarbons on bacteria, plants and animals is correspondingly increased (HOLDGATE 1979). Similarly endangered are areas where mineral oil spills drift onto water or littorals, where colonies of mammals or birds live or breed with often thousands of individuals. The ecological effects of the oil contamination on such affected populations could be devastating. Inland waterways are even more intensely endangered by mineral oil contaminations than sea areas, as the probability there that mineral oil streaks reach ecologically valuable littorals is even much higher (see below).

2. Ecotoxicity of mineral oil contaminations

The ecotoxicological effects of mineral oil contaminations are varied and as a rule difficult to understand. The reasons for this are numerous. Firstly this is due to the fact that mineral oil and mineral oil products due to their physical/chemical composition harm organisms and ecosystems in different ways: physically, chemically and ecologically. In addition, mineral oil products, depending on origin and use, are of different and widely varying chemical compositions as well as varied consistencies. Ultimately it must still be taken into consideration that the ingredients in mineral oils are largely hydrophobic, which is why they do not generally dissolve easily in aqueous phases. Due to this heterogeneity a realistic risk assessment of mineral oil contaminations from a toxicological and ecotoxicological perspective proves extremely difficult.

The **physical effects** of mineral oil contaminations are above all linked with the specific weight and the physical consistency of each product. They are the result of contact with and the wetting of organisms with the mineral oil phase. Agglutination and obstruction of gills and breathing openings are the results, whereby particularly less mobile and sessile animal species are more severely affected. The often observed mass death of marine invertebrates following a massive mineral oil contamination is generally less attributable to the toxic effects of hydrocarbons than to the aforementioned physical effects, which lead to death by suffocation of the affected organisms (ALBERS 1995). Similar effects are to be expected for freshwater invertebrates, whereby the potential risk to them is further increased due to the small size and structuring of the habitats. Also larval stages of fish, which reside in shallow water areas covered with an oil film, are seriously threatened with death by suffocation. Mineral oil slicks also have devastating effects on those animal species, whose temperature regulation is dependent on the coating of hair or feathers on body surfaces. The agglutination and silting of coat and feathers of such animal species lead to death within a short period. Affected by this are practically all types of waterbirds and some mammals, such as sea otters, beavers, arctic seals and polar bears. The frequently observed attempts of the animals to free themselves from mineral oil as a rule soon lead to a state of exhaustion, which is even intensified by the ingestion of mineral oil causing gastrointestinal haemorrhaging (bleeding), which additionally weaken the animals (ALBERS 1995). The particularly high sensitivity of some types of algae (e.g. *Fucus vesiculosus*, *Laminaria digitata*, *Porphyra umbilicalis*) to mineral oil contaminations also appears to be due to a physical effect. In this case extremely thin (< 10 µm thick) oil films on the plant surfaces lead to significant harm to the gas exchange and photosynthesis of the affected types of algae, and drastic population declines are the result (RAMADE 1987).

The **chemical effects** of mineral oil contaminations on organisms can above all be attributed to the **toxicity and ecotoxicity** of hydrocarbon compounds released from the mineral oil and entering the solution. Particularly toxic among these are aromatic hydrocarbons and longer-chained, aliphatic (in part branched) hydrocarbon compounds. As a rule, the toxicity of these substances rises with the increasing score in aromatics and increasing chain lengths for aliphates. Among different mineral oil products kerosene is above all particularly toxic. It is approx. ten times more toxic than crude oil and other refinery products, such as diesel oil or petrol (BETTON 1984).

The situation that some formulations contain up to hundreds of different substances, which are moreover largely hydrophobic, makes testing the toxicity of mineral oil products more difficult. A standardisation of the test parameters under such circumstances is indeed possible yet relatively labour intensive and difficult (BETTON 1984). As a result of this much of the on hand toxicity data on mineral oil products and hydrocarbons released from them can only be compared with one another to a limited degree, which makes ecotoxicological evaluation extremely difficult (BETTON 1984). It has been established however that egg and larvae stages of marine and aquatic organisms in particular are highly sensitive to hydrocarbons from mineral oil products (see **Table 1**). In inland waterways (seas and rivers) this affects above all shallow water in the littoral areas, which are chosen by some species for egg laying, or in which fish are spawned or the larval stages of other animal species reside. Otherwise, mineral oil contaminations have toxic effects on above all zooplanktonic organisms, while many benthic invertebrates appear to be less sensitive. Adult fish also appear to be scarcely affected by acute lethal effects through hydrocarbons from mineral oil products. On the other hand, it has been proven that under chronic contaminations conditions hydrocarbons lead to increased cancer in fish (RAMADE 1987). Some phytoplanktic algae in any event have highly sensitively reactions to acute contaminations by hydrocarbons from mineral oil products.

Table 1: Toxicity (LC_{50}, 96 hrs) of crude oil and soluble hydrocarbons from mineral oil products on various aquatic animal species, including their larval stages (compiled according to BETTON 1984). In most cases toxicity ranges are provided.

Species	Test material	96 hrs LC_{50} (Mg/L)
Fish	mineral oil	88 – 18,000
Fish eggs and larvae	"	0.1 – 100
Benthic crustaceans	"	56
Aquatic macrophytes	soluble hydrocarbons	10 – 100
Eggs and larvae (of all species)	" "	0.1 – 1.0
Gastropods	" "	1 – 100
Mussels	" "	5 – 50
Benthic crustaceans	" "	1 – 10
Other benthic invertebrates	" "	1 – 10

This toxicological effect is however not to be confused with the serious physical impairment of aquatic plants through thin, surface wetting oil films (see above). Already at mineral oil concentrations of 0.2 µg/L the reproduction of some types of phytoplanktic algae (e.g. *Fucus* *edentatus*) can be severely impaired (RAMADE 1987). The primary production of certain phytoplanktic algae is greatly reduced through the toxic effects of mineral oil contaminations, whereby aromatic mineral oil components in particular are responsible for this (RAMADE 1987).

Very little is known about the toxicological effects of mineral oil and mineral oil components on terrestrial soil organisms. In any event there is a little research on this. The conclusion suggested is that negative effects on the reproduction and genetic integrity of the affected organisms is to be anticipated in this area above all under chronic conditions.

Ecological effects of crude oil contaminations are understood to include particularly those, which result in **serious impairments or changes in the habitats** of organisms, so that the organisms are deprived of the basis for life due to indirect effects. This is e.g. the case if the substrate that serves as a base for sessile life forms is no longer inhabitable as a result of the oil slick. In inland waterways and rivers oil slick coatings can destroy the substrate, which serves as egg-laying area for adult individuals of numerous species. Another type of ecological impacts may thereby occur, in that numerous organisms are deprived of basic nutrition due to the mineral oil contamination, because -as a result of the toxicological contamination- fodder plants or marsupials disappear (DALLINGER 1998).

3. Combat and restoration measures

There is longstanding experience on hand regarding the combat of mineral oil contaminations and restoration of contaminated areas in the marine area, where massive mineral oil releases have occurred, above all resulting from repeated tanker accidents in the past 25 years in an order of magnitude of up to 200,000 tonnes per event (RAMADE 1987; FENT 1998).

In the course of combating the oil contamination following the “Torrey Canyon” catastrophe off the British southwest coast in the year 1967 (see above) large surface area detergents were initially used (SMITH 1970). The main motivation for the use of detergents was the probably justified fears that the incidence and visibility of oil slicks and oil streaks on economically significant swimming beaches in the south could damage the thriving beach tourism there. It has been shown thereby that the detergents used, thanks to the reduction of the surface tension at the phase boundary layer between water and oil were indeed capable of transforming the silty water-in-oil emulsion into an oil-in-water emulsion nearly imperceptible to the naked eye. At the same time the bitter experience also had to be made that the detergents used were to a degree more toxic than hydrocarbon compounds stemming from the crude oil in solution. Particularly the combination of detergents with aromatic and aliphatic hydrocarbons led to devastating ecological after-effects for flora and fauna, from which the affected coastal habitats are only recovering slowly and with difficulty (HOLDGATE 1979). It must in any event be pointed out that the surface active product blends used at that time consisting of organic solvents represent a high percentage of aromatic hydrocarbons with anion active and non-ionogenic detergents (SMITH 1970); hence a product, the chemical composition of which would have given rise to the expectation that it would be only slightly toxic or even non toxic. Probably primarily due to this long history of negative bad experiences, detergents are today only used in isolated cases to combat crude oil and mineral oil contaminations. The current conventional measures to combat mineral oil contaminations in marine habitats are as a rule based on procedures consisting of several steps, in which massive oil slicks or oil streaks are initially removed mechanically. In a second phase the remaining contaminations are chemically bound and then also mechanically removed; or attempts are being made to accelerate the biological degradation and hence the disappearance of residual oil impurities through the addition of fertilisers containing nitrogen or phosphorous (ALBERS 1995). In mineral oil contamination of inland waterways, however, the increased use of detergents has been taken into consideration in recent years (see e.g. the broadcast “MODERN TIMES” in ORF 2/10/1998). This is above all of interest with respect to the fact that since the seventies increased surface active substances have been developed based on unbranched anion active detergents (such as linear alkyl benzene sulfonate), which are widely considered to be environmentally compatible, due to their good biological degradability and lower toxicity at correspondingly low concentrations (RAMADE 1987; HENNES-MORGAN u. de OUDE 1994; FENT 1998) (see also **Table 2** regarding this).

Table 2: Toxicity (LC_{50}, 96 hrs) of anionic and non-ionogenic detergents for various marine and aquatic organism groups (compiled acc. to HENNES-MORGAN u. de OUDE 1994 and RAMADE 1987). Toxicity ranges were provided.

Species, Organism group	Anionic detergents (96 hrs LC_{50}, mg/L)	Non-ionogenic detergents (96 hrs LC_{50}, mg/L)
Freshwater algae	1 – 300	---
Marine invertebrates	1 – 800	0.1 – 50
Freshwater invertebrates	1 – 270	1 – 350
Fish	0.05 – 15	1 – 70

Under certain ecological conditions the use of environmentally compatible detergents to combat mineral oil contaminations would be in fact advisable and therefore should be re-evaluated. This applies e.g. in marine areas for use in dealing with saving bird and mammal colonies from oil slicks, the immediate effect on the plumage and fur of the affected individuals causes the failure of temperature regulation and would consequently lead to the death of the animals (see above).

The measured use of environmentally compatible detergents to combat mineral oil contaminations may be advisable, however, in **inland waterways** above all. Mineral oil slicks and oil streaks there may in no event be left to their own devices, as due to the smallness and ecological structuring of the affected habitats the probability is very high that drifting oil streaks soon reach littorals or ecologically valuable, shallow water areas. Any natural degradation processes cannot be effective here without the mineral oil slick and the hydrocarbon compounds contained therein first having caused major injuries. Solely the mechanical removal of oil streaks here is probably not enough. Similar considerations are applicable to contaminated soil, if, as shown below, products are used which promote biological degradation of hydrocarbons in contaminated soil at simultaneously lower toxicity. In any event, however, the combat measures would have to be done in two steps, even given such prerequisites: environmentally compatible, chemical products should basically be used only following prior mechanical removal of a large portion of the mineral oil volume.

4. “Bioversal” as a potential alternative

For more than ten years a product has been on the market, which appears to satisfy the demand for an environmentally compatible preparation for use in combating mineral oil contaminations. According to information from the company “Bioversal Trade and Technologies GmbH” (Haidequerstrasse 1, Vienna) (see information sheet A 1000), “Bioversal” is not a simple detergent but rather a technologically balanced product consisting of several components, which in addition to environmentally compatible, anionic and non-ionogenic detergents contains plant extracts, which act as bioactivators for the degradation of mineral oil components. The manufacturing company has not made public the chemical nature of the individual components for reasons of patent law, yet nevertheless credibly assured that the product contains linear, somewhat degradable, anionic detergents and non-toxic, non-ionogenic detergents, however in no way contains surface-active substances similar to the toxicologically hazardous alkyl phenol polyethoxylate (regarding this see FENT 1998). The distribution company described the effect of “Bioversal” stating that the mineral oil films and streaks are dissolved via the surface active substances contained in the product, whereby small micelles (with a diameter in the order or magnitude of one µm) form, inside of which the existing mineral oil phase is dissolved. In contrast to conventional detergents, the additional non-ionogenic detergents in “Bioversal” lend the micelles formed the ability to climb upwards in the waterbody. A bioactivator consisting of plant extracts in the micelle lining furthermore ensures that these formations known as “biocaps” are very quickly attacked by bacteria and finally completely mineralised together with their content. Unfortunately there is as yet no experimental evidence on this process. The fact is, however, that in laboratory testing “Bioversal” apparently exhibits relatively little acute ecotoxicity and is furthermore capable of accelerating the microbial degradation of anion active detergents and hydrocarbons from mineral oil products and in some cases making this even possible to begin with. An integral, ecotoxicological evaluation on the use of “Bioversal” has been lacking up to now. This should be carried out based on previously available data regarding the biological degradation and toxicity of the product.

5. Ecological and ecotoxicological evaluation of “Bioversal”

An integral, ecotoxicological evaluation of the product “Bioversal” may not however be exclusively oriented to the experimentally determined toxicity data. The test should rather be carried out in consideration of reigning ecological framework conditions in the field and considering all the important “Bioversal” characteristics to undertake a differentiated evaluation.

The estimate of the **ecotoxicity of the product “Bioversal”** in relation to its measured or calculated environmental concentrations following application in the field doubtless plays a pivotal role thereby. **Figure 1** shows that the ecotoxicologically relevant, acute effective concentrations of “Bioversal” for various organism groups depending on framework conditions of the test carried out (LC_{50}/EC_{50}, and/or LC_0/EC_0/NOEC) are in the order or magnitude from 10^2 to 10^5 mg/L. Such high variability of effective concentrations is totally normal and acceptable for toxic substances or groups of toxic substances. It is in any event important that the concentration ranges determined has to be proven by ample independent experimental data. It is also to be noted that the acute toxicity of “Bioversal” is relatively slight. This can be measured by comparing toxicity ranges determined for “Bioversal” with toxicity data for anionic and non-ionogenic detergents (see **Table 2**).

Figure 1: Ecotoxicological evaluation of "Bioversal". The respective concentrations/concentration range (ordinate: mg/L) at which "Bioversal" is effective is specified in the four columns (I – IV). Column I and II show a summary of previous exiting data on the acute ecotoxicity tests for "Bioversal" on various organism groups with each LC_{50} and EC_{50} concentrations observed (Column I), and/or the experimentally determined concentrations at which no effect could be observed (LC_0/EC_0/NOEC) (Column II) [NOEC…"No Observed Effect Concentration"]. Column III shows the "Bioversal" concentrations at corresponding application dilution (%). Column IV shows the "Bioversal" concentrations to be anticipated in the field given the assumption that the corresponding application dilutions (%) admix with the uppermost 10 (a), 20 (b), 30 (c) and/or 50 cm (c) following application in the water over a surface of 200 m^2. The data indexes in Columns 1 and 2 refer to the institutions that have carried out the respective independent tests. These are: 1, 5 ... Gelsenkirchen Hygiene Institute; 2, 3 ... IMU Vienna; 4 ... Ecotest, Czech Republic; 6 ... IWL Cologne; 7 ... EMPA St. Gallen; 8 ... ENEL Ricerca, Milan

For anionic as well as for non-ionogenic detergents the lowest toxicity threshold in each case falls within a concentration range of 0.1 to 1 mg/L, for "Bioversal" on the contrary, this threshold value falls within the concentration range of 10 to 100 mg/L (see **Figure 1**). One explanation for this could be that "Bioversal" in fact contains particularly environmentally compatible anionic and non-ionogenic detergents, which in addition only constitute a certain fractional amount of the volume of the finished products. **Figure 1** furthermore shows that the calculated and anticipated effective concentrations of "Bioversal" in the field are one order of magnitude range below the lowest toxicity threshold. Even with a hypothetical, very high application dilution of "Bioversal" at 10% (as a rule the application dilutions recommended by the distributor amount to 1 to 3%, in exceptional cases up to 7%) the corresponding expected field concentrations are always clearly below the lowest acute toxicity threshold. This confirms the assumption that when applying "Bioversal" to combat mineral oil contaminations in the field no acute ecotoxicological effects are to be anticipated. In fact, when maintaining the application concentrations in the field, nearly all institutions that have been entrusted up to now with a toxicological assessment of the product "Bioversal" (regarding this see **Tables 3 and 4** in the appendix), allocate it to Goods Hazardous to Water, Class 0. The **toxicity experiments** carried out on **mammals** and the dermatologically innocuous findings additionally suggest that the recommended dilution concentrations of "Bioversal" exhibit no harmful effects on humans, which is important for persons entrusted with the application of the product.

The "Bioversal" product description, apart of its relatively low ecotoxicity, also points to the situation that the substances contained in the product are easily biologically degradable under aerobic conditions. The available test results regarding this (see **Table 4** in the appendix) confirm this point of view. Practically all findings by each testing institution document that the product "Bioversal" is **biologically easily degradable** (with degradation rates of up to 99% within 5 to 13 days), and that the ingredients identified in "Bioversal" (total hydrocarbons and anion-active detergents) likewise within 11-30 days (for hydrocarbons) and/or 7 days (for anion active detergents) are subject to a complete biological degradation. The degradation value for anion active detergents in “Bioversal” are hence in the range of those degradation rates that are also authenticated by other authors (see e.g. HENNES-MORGAN u. OUDE 1994). The available data moreover show that “Bioversal”, even at increased concentrations, causes no inhibition of the nitrification so important to the ecological self-cleaning of the water (**Table 4** in the appendix). Moreover, individual institutions were also able to prove that “Bioversal” is in fact capable of **encouraging and accelerating** the **biological degradation** of exogenic hydrocarbons released by mineral oil products (ENEL Ricerca, Italy and IMU Vienna findings see **Table 4** in the appendix). Thus, not only the environmental compatibility of “Bioversal” but also the suitability of applying this product to combat mineral oil contaminations is impressively documented.

The use of “Bioversal” as a means to combat mineral oil contaminations in inland waterways consequently not only appears justified but in most situations even advisable and desirable. As previously mentioned (see above), mineral oil contaminations in our generally small inland waterways can neither be left to their own devices, nor can the mechanical removal of mineral oil slicks and oil streaks alone solve the problem. Following mechanical cleaning, additional, efficient and environmentally compatible cleaning would also be desirable. This is even more relevant as in our highly structured inland waterways the transition zones between water and the surrounding area very often house ecologically valuable habitats, which could also be severely affected by residual oil slick contamination. The use of “Bioversal” appears to be suitable to close these gaps according to all previous existing data.

6. Valuable additional data yet to be determined

The toxicity data on hand refers to the acute ecotoxicity of “Bioversal” in water. No information is on hand regarding the chronic ecotoxicity, however all previous experiences with comparable toxic substances (particularly hydrocarbons and detergents) indicate (see FENT 1998) that the chronic toxicity of such substances as a rule falls within the concentration range of the acutely elevated LC_0 and EC_0, and/or NOEC concentrations. This may also apply to “Bioversal”. In favour of “Bioversal” in this context is the fact that all previously available tests of its degradation behaviour (see **Table 4** in the appendix) point to a rapid and complete, aerobic biological degradation within few days. The question of chronic toxicity in the case of “Bioversal” may therefore be less current than is applicable to comparable, more persistent products. Nevertheless chronic toxicity tests should be carried out.

Although some of the available data compiled on the biological degradation of “Bioversal” also relates to its degradation behaviour in the soil, ecotoxicological data for terrestrial organisms is still fully lacking. Based on previous experiences it can also be cautiously determined here that the toxic effects of a product slightly toxic to aquatic organisms are probably not very toxic to terrestrial invertebrates. However, corresponding trials are still to be carried out in this respect as well.

The question also remains open regarding potential synergistic interactions of “Bioversal” in combination with the hydrocarbons stemming from mineral oil contaminations. The only test (luminescent bacteria test) which addresses this question (by the company ENEL-Ricerca, see **Table 3** in the appendix), indicates potential synergistic interactions of “Bioversal” in combination with increased mineral oil contaminations, in any event with enormously high concentrations of “Bioversal” in the region of 25,000 mg/L, as they are not to be expected after application of the product under field conditions (see **Figure 1**). However the test result still indicates that temporal components could thereby play a certain role, whereby synergistic effects above all at the beginning of the application at enormously high concentrations of “Bioversal” cannot be entirely excluded, which however – probably due to the favourable degradation behaviour of “Bioversal” – drop with time below the effect threshold of the control value. There remains the need for more research in this area as well.

A further point requiring analytical clarification is “Bioversal” concentration applications actually occurring in the field. As seen in the legend of **Figure 1**, the field concentrations provided for “Bioversal” in any event represent realistic estimates, which were calculated from information and data on “Bioversal” application. For reasons of caution these values are still over-estimated so an analytical clarification of the concentrations occurring in the field after “Bioversal” application could adjust these concentrations even lower.

7. Recommendations to improve product acceptance and accompanying measures to optimise the use of “Bioversal”

Product acceptance for “Bioversal” can certainly be further improved by additional tests and analyses (see above). Moreover it appears that a recommended and responsible use of “Bioversal” in the field is only possible after appropriately training the operating personnel (fire brigades and other relief units). It is hence e.g. essential to consider the ecological nature of the water (wetland, pool, sea etc. ?) and the volumes of waterbody available for dilution prior to using “Bioversal”. It is therefore advisable to link “Bioversal” application with the condition of the appropriate, ecological training.

8. Literature

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9. Additional documents

INFORMATION SHEET A 1000 (“Bioversal” QF”) of the company “Bioversal” Trade and Technologies GmbH” (Vienna, Haidequerstrasse).

MODERN TIMES, 2/10/1998: ORF 2 broadcast on the use of “Bioversal” on the occasion of a mineral oil contamination on the Donau river.

Summary

With a **global emission of 88 million tonnes per year** the contamination of ecosystems with **hydrocarbons** from mineral oil and mineral oil products represents a worldwide problem, which impacts marine ecosystems in particular. 35% of these global emissions effect continental/terrestrial and aquatic (freshwater) ecosystems. Sources of contamination coming into question here are motor vehicle traffic in the broadest sense, shipping traffic, air traffic, continental oil drilling facilities, refineries and their infrastructure, waste incineration facilities and waste disposal sites, as well as all other consumers of fossil energy sources. A particular problem is represented by massive contaminations with large volumes of mineral oil or mineral oil products (crude oil, petrol, diesel oil, fuel oil, etc.), which can mainly enter the soil and water undiluted due to accidents or unintentional release.

Mineral oil and mineral oil products, due to their physical/chemical nature, can harm organisms and ecosystems in various ways. **Physical effects** of mineral oil contaminations depend above all on the specific weight and the physical consistency of the respective product. These result from contact and wetting organisms with the mineral oil phase. **Chemical effects** of mineral oil contaminations on organisms can essentially be attributed to the **toxicity and ecotoxicity** of the hydrocarbon compounds released from the mineral oil and added to the solution. **Ecological effects** of mineral oil contaminations are ultimately understood to be those, which result in **serious harm or changes to the habitats** of organisms, such that the latter is deprived of the basis for life.

Under certain ecological prerequisites the use of **special environmentally compatible detergents** in combination with mechanical cleaning measures to combat mineral oil contaminations would be sensible and consequently should be **re-evaluated**. This applies above all to mineral oil contaminations in **inland waterways**. Mineral oil slicks and oil streaks there should in no event be left to their own devices, as due to the smallness and ecological structuring of the affected habitats the probability is very high that drifting oil streaks soon hit littorals or reach ecologically valuable, shallow water areas. Any naturally occurring degradation processes could not be effective here without the mineral oil slick and hydrocarbon compounds contained therein first causing major damages.

For more than ten years a product described as **“Bioversal”** has been on the market that appears to satisfy the demand for an **environmentally friendly preparation**, which can be used to combat mineral oil contaminations. An integral **ecotoxicological evaluation** on the use of “Bioversal” is still lacking. This should be done subsequently based on previously available data on the biological degradation and toxicity of the product. With the assistance of data obtained experimentally on acute ecotoxicity it was documented that “Bioversal” exhibits a **comparably low toxicity**, and that moreover its active ingredients under aerobic conditions are **completely biologically degradable** within a few days. Moreover “Bioversal” encourages the biological degradability of mineral oil components. The use of “Bioversal” as a means to combat mineral oil contaminations hence appears to be ecologically compatible and expedient.

APPENDIX: Table 3: Toxicity test by various institutions and companies of the product "Bioversal" carried out for different organism groups with information of test authority, date, test material concentration, short test description with guidelines as well as any test results

Organism	Test authority, Test Institute	Date	Test material (Concentration)	Test description (methodology)	Guidelines	Results
<u>**Bacteria:**</u>						
Activated sludge organisms	Gelsenkirchen Hygiene Inst.	19/3/1994	"Bioversal" (undiluted)	No information	No information	$EC_0 \leq 600$ mg/L
Activated sludge organisms	IMU Vienna	8/9/1999	"Bioversal" QF (0.2%)	Bacteria inhibition BSB inhibition (mg/L)	Austrian standard ORM B 5105	At 0.2% (2 g/L) No inhibition observed
Activated sludge Organisms	IMU Vienna	8/9/1999	"Bioversal" HC (0.1 - 0.3%)	Nitrification inhibition 20°C, in darkness	Austrian standard EN ISO 9509	EC_{50} (incubation): > 0.3% Corresponds to EC_{50} > 3 g/L
			"Bioversal" QF (0.1 - 0.3%)	Nitrification inhibition 20°C, in darkness	Austrian standard EN ISO 9509	EC_{50} (incubation): > 0.2-0.3% Corresponds to EC_{50} > 2-3 g/L
***Pseudomonas* sp.**	Gelsenkirchen Hygiene Inst.	11/11/1997	"Bioversal" HC (10%)	Cell proliferation Inhibition test, 16 hrs	DIN 38412 L8	EC_{10} (16 hrs): > 800 g/L EC_{50} (16 hrs): > 800 g/L No inhibition observed
			"Bioversal" HC (Undiluted)		Conversion: Undiluted	EC_{10} (16 hrs): > 80 g/L EC_{50} (16 hrs): > 80 g/L No inhibition observed
Pseudomonas sp.	Gelsenkirchen Hygiene Inst.	11/11/1997	"Bioversal" QF (6%)	Cell proliferation Inhibition test, 16 hrs	DIN 38412 L8	EC_{10} (16 hrs): > 800 g/L EC_{50} (16 hrs): > 800 g/L No inhibition observed
			"Bioversal" QF (undiluted)		Conversion: Undiluted	EC_{10} (16 hrs): > 384 g/L EC_{50} (16 hrs): > 384 g/L No inhibition observed
Vibrio fischeri	ENEL Ricerca	20/5/1999	"Bioversal" HK (2.5%) in aqueous solution	Bioluminescence inhibition test (Microtox)	"Microtox" v. Microbios Corporation, Carlsbad, CA (USA)	**Effective concentration (EC_{50}) extremely high: 25,000 mg/L!**
			"Bioversal" HK (2.5%) in soil suspension			**Effective concentration (EC_{50}) extremely high: 25,000 mg/L!**

Continuation: Table 3

Algae:						
Scenedesmus ***Subspicatus***	Gelsenkirchen Hygiene Inst.	11/11/1997	"Bioversal" HC (10%)	Inhibition effect of the cell proliferation 23°C, 8000 Lux over 72 hrs.	OECD guidelines 201	EC_{10} (0 to 72 hrs): 4500 mg/L EC_{50} (0 to 72 hrs): 7500 mg/L
			"Bioversal" HC (Undiluted)			EC_{10} (0 to 72 hrs): 450 mg/L EC_{50} (0 to 72 hrs): 750 mg/L
Scenedesmus ***Subspicatus***	Gelsenkirchen Hygiene Inst.	11/11/1997	"Bioversal" QF (6%)	Inhibition effect of the cell proliferation 23°C, 8000 Lux over 72 hrs.	OECD guidelines 201	EC_{10} (0 to 72 hrs): 14000 mg/L EC_{50} (0 to 72 hrs): 16900 mg/L
			"Bioversal" QF (Undiluted)			EC_{10} (0 to 72 hrs): 840 mg/L EC_{50} (0 to 72 hrs): 1014 mg/L
Scenedesmus ***Subspicatus***	ECOTEST company, Czech Republic	19/6/2000	"Bioversal" HC (Undiluted)	Inhibition effect of the cell proliferation 23°C, 7000 Lux over 72 hrs.	Pursuant enclosure no. 2 Decree no. 299/98 Slg.	EC_{50} [growth speed.] (0-72 hrs.): > 100 mg/L EC_{50} [bio mass] (0 - 72 hrs.): 98.97 mg/L
			"Bioversal" QF (Undiluted)			EC_{50} [growth speed] (0 - 72 hrs.): > 100 mg/L EC_{50} [bio mass] (0 - 72 hrs.): 64.65 mg/L

Continuation: Table 3

<u>Invertebrates:</u>						
Daphnia magna **STRAUS**	Gelsenkirchen Hygiene Inst.	11/11/1997	"Bioversal" HC (10%)	Immobility test 20°C, 48 hrs.	OECD guidelines 202	EC_0 (48 hrs): 10000 mg/L EC_{50} (48 hrs): 14000 mg/L EC_{100} (48 hrs): 20000 mg/L
			"Bioversal" HC (Undiluted)		Conversion: Undiluted	EC_0 (48 hrs): 1000 mg/L EC_{50} (48 hrs): 1400 mg/L EC_{100} (48 hrs): 2000 mg/L
Daphnia magna **STRAUS**	Gelsenkirchen Hygiene Inst.	11/11/1997	"Bioversal" QF (6%)	Immobility test 20°C, 48 hrs.	OECD Guidelines 202	EC_0 (48 hrs): 2000 mg/L EC_{50} (48 hrs): 4500 mg/L EC_{100} (48 hrs): 7000 mg/L
			"Bioversal" QF (Undiluted)		Conversion: Undiluted	EC_0 (48 hrs): 120 mg/L EC_{50} (48 hrs): 270 mg/L EC_{100} (48 hrs): 420 mg/L
Daphnia magna **(Own stock)**	ECOTEST, Czech Republic	19/6/2000	"Bioversal" HC (Undiluted)	Immobility test 21°C, 48 hrs.	Pursuant enclosure no. 2 Decree no. 299/98 Slg.	EC_0 (48 hrs): 50 mg/L EC_{50} (48 hrs): 65.11 mg/L
			"Bioversal" QF (Undiluted)			EC_{50} (48 hrs): > 100 mg/L

Continuation: Table 3

<u>Fish:</u>						
Golden orfe (*Leuciscus idus*)	Gelsenkirchen Hygiene Inst.	19/3/1994	"Bioversal" (undiluted)	Acute fish toxicity	No information	$LC_0 \leq 125$ mg/L
Golden orfe (*Leuciscus idus*)	Gelsenkirchen Hygiene Inst.	11/11/1997	"Bioversal" HC (10%)	Acute fish toxicity 20°C, 48 hrs.	DIN 38412 Part 15	LC_0 (48 hrs): 2500 mg/L LC_{50} (48 hrs): 3000 mg/L LC_{100} (48 hrs): 3400 mg/L
			"Bioversal" HC (Undiluted)		Conversion: Undiluted	LC_0 (48 hrs): 250 mg/L LC_{50} (48 hrs): 300 mg/L LC_{100} (48 hrs): 340 mg/L
Golden orfe (*Leuciscus idus*)	Gelsenkirchen Hygiene Inst.	11/11/1997	"Bioversal" QF (6%)	Acute fish toxicity 20°C, 48 hrs.	DIN 38412 Part 15	LC_0 (48 hrs): 4000 mg/L LC_{50} (48 hrs): 4500 mg/L LC_{100} (48 hrs): 5000 mg/L
			"Bioversal" QF (Undiluted)		Conversion: Undiluted	LC_0 (48 hrs): 240 mg/L LC_{50} (48 hrs): 270 mg/L LC_{100} (48 hrs): 300 mg/L
Guppies (*Poecilia reiticulata*)	ECOTEST, Czech Republic	19/6/2000	"Bioversal" HC (Undiluted)	Acute fish toxicity 22°C, 96 hrs.	Pursuant enclosure no. 2 Decree no. 299/98 Slg.	LC_{50} (48 hrs): > 100 mg/L LC_{50} (96h): > 100 mg/L
			"Bioversal" QF (Undiluted)			LC_{50} (48 hrs): > 100 mg/L LC_{50} (96h): > 100 mg/L
<u>Mammals:</u>						
Rats	Gelsenkirchen Hygiene Inst.	19/3/1994	"Bioversal" (undiluted)	Acute mammal toxicity	No information	LD_{50}: > 2000 mg/kg (hrs ?)
Rats	Gelsenkirchen Hygiene Inst.	11/11/1997	"Bioversal" HC (10%)	Acute mammal toxicity Dose: > 2000 mg/kg	KZ 20307 ("Limit Test") (Umw.Bu.Amt)	LD_{50}: > 2000 mg/kg
			"Bioversal" (undiluted)			LD_{50}: > 200 mg/kg
			"Bioversal" QF (6%)			LD_{50}: > 2000 mg/kg
Rats	Gelsenkirchen Hygiene Inst.	11/11/1997	"Bioversal" (undiluted)	Acute mammal toxicity Dose: > 2000 mg/kg	KZ 20307 ("Limit Test") (Umw.Bu.Amt)	LD_{50}: > 120 mg/kg
Rabbits (albino)	Gelsenkirchen Hygiene Inst.	13/1/1998	"Bioversal" (undiluted)	Irritating effect on the eye	Guidelines of the commission 92/69/EWG	Does not irritate the eye
Human	Derma Consult GmbH	27/6/1997	"Bioversal" (undiluted)	Main compatibility	GLP guidelines	harmless

APPENDIX: Table 4: Tests carried out by different institutions and companies on the biological degradation behaviour of the product "Bioversal" for various toxic substance groups (particularly anion active detergents and hydrocarbons) with information by test authority, date, test material concentration, brief test description with guidelines, as well as any test results

Test principle (Micro organisms)	Test authority, Test institute	Datum	Test material (Concentration)	Test description (Methodology)	Guidelines	Results
<u>Inhibition test:</u>						
Inhibition of the <u>BSB_5 degradation</u> (activated sludge)	IWL Cologne	24/11/1989	"Bioversal" in doubled application conc. (40 ml/L = 4%)	Inhibition of the BSB_5 degradation (Aqualytik AL 214)	Austrian standard ORM B 5104 in connection with the Salzburg guidelines - Draft	BSB_5 value of test waste water with "Bioversal": 128 mg/L **Result: no inhibition of the BSB_5 degradation**
<u>Nitrification</u> inhibition (activated sludge)	EMPA St. Gallen	24/10/1991	"Bioversal" diluted (690 mg/L = 0.069%)	Determining the ammonium conc. using the Berthelot method (Initial content at NH_4 N: 6.5 mg/L)	Method 30 of the EDI guidelines	Final content of NH_4-N (after 14 days): < 0.1 mg/L **Result: No impairment to the nitrification**
<u>Nitrification</u> inhibition (activated sludge)	IMU Vienna	19/10/1999	"Bioversal" HC (0.1%, 0.2%, 0.3% = 1 ml/L, 2 ml/L, 3 ml/L)	4-hour incubation, incl. determining ammonium N with the assistance of the capillary electrophoresis	Austrian standard EN ISO 9509	**EC_{50}: > 0.3%**
			"Bioversal" QF (0.1%, 0.2%, 0.3% = 1 ml/L, 2 ml/L, 3 ml/L)			EC_{50}: = 0.2 - 0.3%

Continuation: Table 4						
<u>Biological degradation</u> **<u>Biological degradation by determining the BSB_5 value</u>** **(activated sludge)**	Gelsenkirchen Hygiene Inst.	19/3/1994	"Bioversal" undiluted in aqueous activated sludge suspension (concentration ?)	Miti Test	OECD – 301 C	Biochemical degradation after 5 days: 73.8%. Biochemical degradation after 28 days: 98%. **Result: Biol. degradability: very good**
			"Bioversal" diluted (1:25 = 40 g/L)			Biochemical degradation after 5 days: 73.8%. Biochemical degradation after 28 days: 98%. **Result: Biol. degradability: very good**
			"Bioversal" diluted (1:100 = 10 g/L)			Biochemical degradation after 5 days: 73.8%. Biochemical degradation after 28 days: 98%. **Result: Biol. degradability: very good**
<u>Biological degradation by determining the BSB_5 value</u> **(activated sludge)**	Gelsenkirchen Hygiene Inst.	22/8/1997	"Bioversal" HC (1 ml in 1 L activated sludge suspension) (corresponds to 0.1%)	Manometric determination of the BSB_5 with CSB (chemical oxygen content) as calculation size	Miti Test OECD – 301 C	Biochemical degradation after 5 days: 87%. Biochemical degradation after 12 days: 98%. **Result: Biol. degradability: very good**
			"Bioversal" QF (1 ml in 1 L activated sludge suspension) (corresponds to 0.1%)			Biochemical degradation after 5 days: 66%. Biochemical degradation after 13 days: 98%. **Result: Biol. degradability: very good**
<u>Biological degradation by determining the BSB_5 value</u> **(activated sludge)**	Gelsenkirchen Hygiene Inst.	11/11/1997	"Bioversal" (10% solution)	Manometric determination of the BSB_5 with CSB (chemical oxygen condition) as calculation size	Miti Test OECD – 301 C	Biochemical degradation after 5 days: 66%. Biochemical degradation after 12 days: 98%. **Result: Biol. degradability: very good**

Continuation: Table 4						
Biological <u>degradation by determining the BSB_5 value</u> **(activated sludge)**	Gelsenkirchen Hygiene Inst.	11/11/1997	"Bioversal" QF (6% solution)	Manometric determination of the BSB_5 with CSB (chemical oxygen condition) as calculation size	Miti Test OECD – 301 C	Biochemical degradation after 5 days: 87%. Biochemical degradation after 11 days: 98%. **Result: Biol. degradability: very good**
Biological <u>degradation by determining the dissolved organic hydrocarbon (DOC)</u> (activated sludge)	EMPA St. Gallen	24/10/1991	"Bioversal" diluted (690 mg/L = 0.069%)	Determination of the DOC (initial content of DOC: 48.3 mg/L)	OECD guidelines 302 B Labour regulation EMPA/EAWAG	Final DOC content (after 28 days, with corrected check value): 1.3 mg/L) **Result: slightly biologically degradable**
Biological <u>degradation of aliphatic hydrocarbons</u> (activated sludge)	Gelsenkirchen Hygiene Inst.	19/3/1994	"Bioversal" undiluted. in aqueous activated sludge suspension (concentration ?)	Determining the aliphatic hydrocarbons (initial content: no information)	No information	Elimination rate aliphatic hydrocarbons after 30 days: approx. 95%. **Result: Biol. degradability: very good**
Biological <u>degradation of hydrocarbons</u> (traditional diesel oil) (activated sludge)	Gelsenkirchen Hygiene Inst.	22/8/1997	"Bioversal" HC (1 ml in 1 L activated sludge suspension) (corresponds to 0.1%) with 870 mg diesel oil	Quantitative determination of hydrocarbons	DIN 38 409 Part 18	Elimination rate of hydrocarbons after 13 days: > 99%. **Result: Biol. degradability: very good**
			"Bioversal" QF (1 ml in 1 L activated sludge suspension) (corresponds to 0.1%) with 870 mg diesel oil	Quantitative determination of hydrocarbons	DIN 38 409 Part 18	Elimination rate of the hydrocarbons after 11 days: > 99%. **Result: Biol. degradability: very good**
Biological <u>degradation of hydrocarbons</u> (oil, kerosene) (activated sludge)	ENEL Ricerca, Italy	20/5/1999	"Bioversal" HK (2.5%) in soil suspension or in water	Determining hydrocarbons after sample restriction via weight analysis 20 days, aerobic, 25-28°C	No information	Hydrocarbon degradation after 20 days: soil suspension: without "Bioversal" 43% with "Bioversal" 84%. Water tests: without "Bioversal" 5% with "Bioversal" 53%. **Result: Promoting the biodegradation v. hydrocarbons very good**

Continuation: Table 4						
Degradation rate of the <u>anion active detergents</u> (activated sludge)	Hygiene Inst. Gelsenkirchen	3/3/1994	"Bioversal" diluted (1 ml/L = 0.1%)	Determining the content of anion active detergents (Initial content: 2.8 mg/L)	No information	Final content of anion active detergents (after 7 days): < 0.05 mg/L. Elimination rate: 99% **Result: Biol. degradability: v. anion active detergents after 7 days closed**
			"Bioversal" HKS diluted (1 ml/L=0.1%)	Determining the content of anion active detergents (Initial content: 1.6 mg/L)	No information	Final content of anion active detergents (after 7 days): < 0.05 mg/L. Elimination rate: 98% **Result: Biol. degradability: v. anion active detergents after 7 days closed**
Degradation rate of the <u>anion active detergents</u> (activated sludge)	Hygiene Inst. Gelsenkirchen	19/3/1994	"Bioversal" undiluted in aqueous activated sludge suspension (concentration ?)	Determining the content of anion active detergents (Initial content: no data)	No information	Elimination rate after 7 days: > 99% **Result: Biol. degradability: v. anion active detergents after 7 days closed**
Degradation rate of the <u>anion active detergents</u> (aerobic, polyvalent micro organisms)	Hygiene Inst. Gelsenkirchen	22/8/1997	"Bioversal" HC (1 ml in 1 L activated sludge suspension) (corresponds to 0.1%)	Determining the content of anion active detergents in creep test (Initial content: 5 mg/L)	DIN 38 409 Part 23-1	Final content of anion active detergents (after 7 days): < 0.05 mg/L. Elimination rate: 99% **Result: Biol. degradability: v. anion active detergents after 7 days closed**
			"Bioversal" QF (1 ml in 1 L activated sludge suspension) (corresponds to 0.1%)	Determining the content of anion active detergents in creep test (Initial content: 6.1 mg/L)	DIN 38 409 Part 23-1	Final content of anion active detergents (after 7 days): < 0.05 mg/L. Elimination rate: 99% **Result: Biol. degradability: v. anion active detergents after 7 days closed**

ADDITIONAL EXPERT REPORT

on the

ecotoxicological evaluation of "Bioversal"

under special consideration of synergistic effects between

"Bioversal" and hydrocarbons

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1. Prerequisites

The company “Bioversal Trade and Technologies GmbH” (Haidequerstrasse 1, Vienna) has for some years been offering “Bioversal”, a product for the decontamination of mineral oil contaminations in soil and water. According to manufacturer information “Bioversal” is a technologically balanced product consisting of several components that, in addition to environmentally compatible, anionic and non-ionogenic detergents, also contains plant extracts, which act as bioactivators for the degradation of mineral oil components. In an expert report prepared in August 2000 (DALLINGER 2000) it was determined that the product (Bioversal HC®) offered by the company “Bioversal Trade and Technologies GmbH” could in fact represent a suitable alternative for combating mineral oil and hydrocarbon contaminations in soil and water due to its environmentally friendly behaviour and, when applied professionally, its reduced ecotoxicity. Such types of contaminations can always occur as a result of accidents or careless handling of mineral oil products and represent a potential danger to our small structured water habitats and soil ecosystems (see DALLINGER 2000). The use of “Bioversal” in such types of contaminations must always be following the prior, primarily mechanical removal of the major portion of the mineral oil residues. Pursuant the manufacturer’s recommendation “Bioversal” should thereby be used in an application dilution of a maximum of 1-3% (in exceptional cases up to 7%) for environmentally gentle decontamination of any remaining residual mineral oil contaminations. The aforementioned expert report (DALLINGER 2000) determined that the effective concentrations of “Bioversal” for different groups of organisms depending on framework conditions of the test carried out (LC_{50}/EC_{50}, and/or LC_0/EC_0/NOEC) are within an order of magnitude range from 100 to 100,000 mg/L. When applying the aforementioned application concentrations in the field according to regulations “Bioversal” concentrations effective in the waterbody are expected to be in the range of 1-5 mg/L (at 1% dilution), 3-15 mg/L (at 3% dilution), and/or 7-25 mg/L (at 7% dilution). In any event the anticipated concentrations in the field were significantly below those threshold values, which exhibited an effect following acute contamination (see DALLINGER 2000). Although there is currently no information on the chronic ecotoxicity of “Bioversal”, all previous experiences with comparable substances (particularly hydrocarbons and detergents) indicate that its chronic toxicity is in the concentration range of the acutely elevated LC_0 and EC_0, and/or NOEC concentrations. This may also apply to “Bioversal” (DALLINGER 2000).

Despite the generally favourable findings on the ecotoxicity of “Bioversal” in previous expert reports some problem areas were addressed that still remain open, the clarification of which in the expert’s opinion would contribute towards additionally increasing the credibility of the environmentally compatible product “Bioversal”. One of the most pressing questions in this context regarded the potential synergistic interactions of “Bioversal” in combination with the hydrocarbons stemming from mineral oil contaminations (see DALLINGER 2000).

The company “Bioversal Trade and Technologies GmbH” has commendably recognised this still existing gap and commissioned two independent institutions with the execution of ecotoxicological tests, the aim of which is to determine if there are any synergistic interactions between “Bioversal” and hydrocarbons coming from mineral oil residues in the water, based on different test procedures. Moreover, a test was commissioned, which was to address the question of the phase distribution of “Bioversal” and hydrocarbon residues in a closed aquatic system after the combined application of “Bioversal” with hydrocarbons. Two institutions were commissioned with these tests: the Interuniversitäres Forschungsinstitut für Agrarbiotechnologie in Tulln [*Interuniversity Research Institute for Agrarian Biotechnology*] (IFA Tulln, Konrad Lorenz Strasse 20, A - 3430 Tulln, Lower Austria) with toxicity tests for luminescent bacteria, algae and daphnia, as well as with the tests on the aforementioned phase distribution; and Forschungszentrum Seibersdorf [*Seibersdorf Research* Centre] (Toxicology Division, A - 2444 Seibersdorf, Lower Austria) with toxicity tests for fish.

The research from the commissioned tests is now on hand (FRITZ 2001 a; FRITZ 2001 b; FENZL et al. 2001 a; FENZL et al. 2001 b). The aim of this additional expert report provided is to subject the ecotoxicity of “Bioversal” in consideration of this additional data on the synergistic effect and on the phase distribution to any necessary re-evaluation.

Synergistic effects in the combined effect of “Bioversal” and hydrocarbon residues

In anticipation of the research carried out it was observed that synergistic interactions of “Bioversal” in combination with mineral oil residues in nearly all test organisms were negligible to slight. The corresponding IFA Tulln (FRITZ 2001 a) and Seibersdorf research centre reports (FENZL et al. 2001 a; FENZL et al. 2001 b) are briefly summarised and explained below.

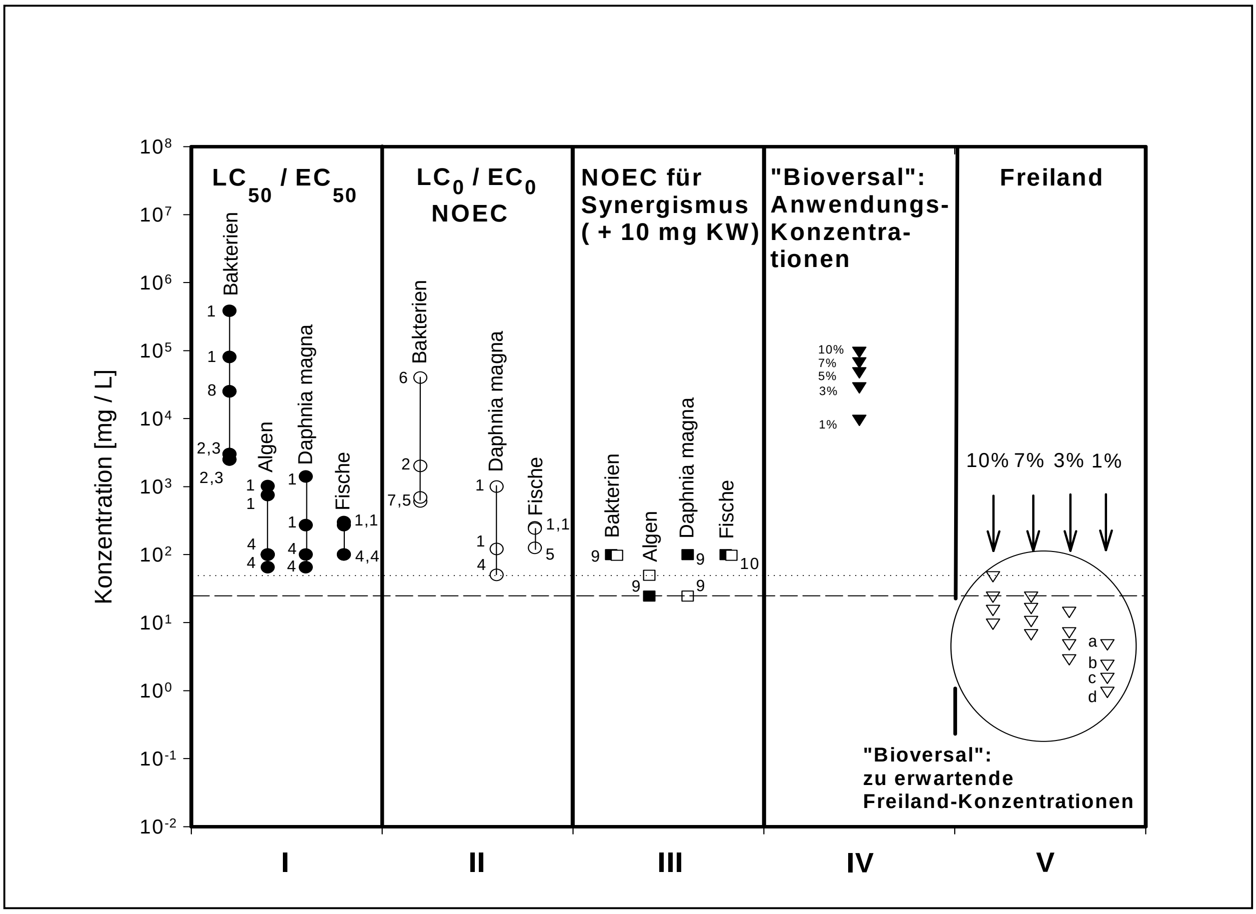
The ecotoxicity tests on the synergistic effect for bacteria, algae and daphnia were carried out by the IFA Tulln, whereby two of the “Bioversal” products available on the market (Bioversal FW® and Bioversal QF®) were tested. Bioversal FW® (corresponds to the product Bioversal HC® from the previous expert report; see DALLINGER 2000) is designed as a product, which is admirably suited for use in the decontamination of mineral oil residues on traffic surfaces and in water, while Bioversal QF® is meant to be used as a fire extinguishing agent. Both products contain the same basic components – albeit at different dilutions. A customary luminescence test with *Vibrio fischeri* was used as a bacteria test carried out pursuant DIN 38412. For algae an acute growth inhibition test was performed with *Selenastrum capricornutum*, corresponding to DIN 38412 L33 and the guidelines of the OECD 201 method. The daphnia test used *daphnia magna* from IFA Tulln’s own stock, and this was tested pursuant DIN 38412 L30 and the OECD 202 guidelines method (FRITZ 2001 a).

The acute ecotoxicity tests on the synergistic effect of Bioversal FW® and Bioversal QF® for fish were performed at the Österreichisches Forschungszentrum Seibersdorf [*Austrian Research Centre, Seibersdorf*] using zebra danio (*brachydanio rerio*) pursuant the guidelines of the EEC Commission Directive 92/69 C.1 (29 December 1992) and the OECD “Good Laboratory Practice” guidelines (FENZL et al. 2001 a; FENZL et al. 2001 b).

Interpreting the results with regard to synergistic tests for luminescent bacteria, algae and daphnia is insofar not easy, as in the majority of cases the respective inhibition curves with increasing concentrations of the components used (Bioversal FW® and/or Bioversal QF® on the one hand and mineral oil residues on the other) run discontinuously, whereby at lower and very high blend concentrations of the two components phases a more powerful inhibition of the test organisms was in part achieved than with intermediary concentrations. In some cases this gave the impression as if there was an intermediary concentration range of the blend of both components, at which the toxic effect is the lowest. This is shown e.g. clearly in the algae test with Bioversal FW®, in which the combinations between “Bioversal” in the concentration range of 6 to 25 mg/L at constant concentration of 10 µl/L diesel show lesser synergistic effects than each higher or lower “Bioversal” concentration in combination with 10 µl/L diesel (FRITZ 2001 a). Similar, if not always so pronounced, inhibition processes are also exhibited with Bioversal QF® for algae and daphnia. An interpretation of this result is in view of the singleness of the test (and hence in absence of existing repetitions) scarcely possible so that the impression that this deals with intermediary blend concentrations between the components, which reduce the synergistic effect and hence the ecotoxicity of the blend, must remain speculative. The reasons for this type of discontinuous run of the inhibition curves could lie in physical/chemical interactions between the tenside components of “Bioversal” and the mineral oil residues. Such types of interactions could for example lead to variable, from the respective concentration of the components concerned dependent phase distributions of the hydrocarbon fractions in aqueous solution (and/or suspension). To examine these types of effects, following the conclusion of the ecotoxicity tests at IFA Tulln, a phase distribution study was also carried out in combination, between “Bioversal” and diesel oil and/or lubricants (regarding this see FRITZ 2001 b). Results of this study are further addressed below.

Regardless of any potential phase distribution problems of the components used, the toxicity tests with microorganisms and daphnia furthermore also showed that only in some cases the so-called EC_{50} values for the respective inhibiting effect were achieved, as a result of the generally flat run of the inhibition curves. This circumstance also made an interpretation difficult so that it was hard under all the specified prerequisites to estimate those threshold concentrations of Bioversal FW® and Bioversal QF® that had no negative impacts on the exposed organisms in combination with mineral oil residues (the conversation here is about “NOEC” concentrations, whereby “NOEC” stands for “No Observed Effect Concentration”). To prevent any erroneous interpretations here, the extrapolation of the NOEC values for microorganisms and algae from the graphics provided (FRITZ 2001 a) is only to be undertaken following consultation and in agreement with the investigator (Dr. Johann FRITZ).

On the other hand, interpreting the synergistic effect of “Bioversal” (Bioversal FW® and Bioversal QF®) in combination with hydrocarbon residues on fish (*Brachydanio rerio*) was not difficult. In this case the interpretation was made in discussion and in agreement with the investigator (Ms. Mag. Christine FENZL) at Seibersdorf Research Centre. Clearly here, as this was also determined in the corresponding report (see FENZL et al. 2001 a; FENZL et al. 2001 b), no synergistic effect between Bioversal FW® and/or Bioversal QF® and the mineral oil residue used could be determined with any of the concentrations tested.



[*Vertically in left margin*]
Concentration (mg/L)

[*Translation of graph above]*

LC_{50}/EC_{50}	LC_0/EC_0 NOEC	NOEC for synergism (+ 10 mg KW)	"Bioversal" application concentrations	Field
Bacteria	Bacteria	Bacteria	10%	10%
Algae	Algae	Algae	7%	7%
Daphnia magna	Daphnia magna	Daphnia magna	6%	6%
Fish	Fish	Fish	3%	3%
			1%	1%

"Bioversal": anticipated field concentrations

Figure 1: Ecotoxicological analysis of "Bioversal". Each concentration/concentration range (Ordinate: mg/L) is specified in the five columns (I – V), at which "Bioversal" (Bioversal HC® and/or Bioversal FW® and Bioversal QF®) has an effect. Columns I and II show a summarisation of existing data on acute ecotoxicity tests for "Bioversal" with various groups of organisms with the respective determined LC_{50} and EC_{50} concentrations (Column I) and/or the experimentally determined concentrations at which no effect could be determined (LC_0/EC_0/NOEC) (Column II) [NOEC ... "No Observed Effect Concentration"]. Column III shows those Bioversal FW® concentrations (black symbols) and/or Bioversal QF® concentrations (white symbols), which combined with 10 µl/L diesel ("loading rate") exhibit no inhibiting effect on the organisms concerned (NOEC for the synergistic effect). Column IV shows the concentrations of "Bioversal" with corresponding initial dilution (%). Column V shows the "Bioversal" concentrations anticipated in the field under the assumption that the corresponding application dilutions (%) admix following application in the water over a surface of 200 m^2 with the uppermost 10 (a), 20 (b), 30 (c) and/or 50 cm (c) of the water column. The data indexes in columns 1 through 3 refer to the institutes that have carried out the respective independent tests. There are: 1, 5 ... Gelsenkirchen Hygiene Institute; 2, 3 ... Vienna IMU; 4 ...Ecotest, Czech Republic; 6 ... IWL Cologne; 7 ... EMPA St. Gallen; 8 ... ENEL Ricerca, Milan; 9 ... IFA Tulln (FRITZ 2001 a); 10 ... Seibersdorf Research Centre (FENZL et al. 2001 a; FENZL et al. 2001 b). More precise information on the synergistic test results is available in **Table 1** in the appendix. The horizontal lines (dotted line: expert reports

DALLINGER 2000; dashes: additional expert reports on hand) show the threshold area of Bioversal concentration at which no effect can be observed.

To facilitate a comparison with existing data and to derive conclusions resulting from it, the results of the synergistic tests were integrated into a graph, the modified form of which has already been represented in the first expert report (DALLINGER 2000). It was thereby shown that due to the in fact lesser, however existing synergistic interactions of the threshold value for the still tolerable “Bioversal” concentration in the field compared to the first expert report (DALLINGER 2000) a further reduction by a quarter decimal power would have to be made. In light of this new interpretation, “Bioversal” dilutions for application in the field hence appeared permissible only in the range of 1-3%, no longer 7% however (see expert report **Figure 1** and **Table 1** in the appendix).

Research on the phase distribution of diesel oil and lubricant residues in aqueous suspension in the presence of Bioversal FW®

As already mentioned above, certain discontinuities in the runs of the inhibition curves for luminescent bacteria, algae and daphnia indicate that, regardless of the actual toxic effect, there are also possible physical/chemical effects, which – depending on blend concentration of the components concerned – lead to different phase distributions and hence to different availabilities of the hydrocarbon fractions in aqueous solution and/or in suspension. In order to examine this phenomenon more closely IFA Tulln carried out a pilot study (FRITZ 2001 b) intended to test whether and to what extent the recovery and distribution of mineral oil fractions without impact, as well as in the presence of various concentrations of “Bioversal”, influence various segments of the waterbody. Two different types of mineral oil (diesel oil and lubricants) in alternating concentrations (0.5 and 10 µl/L) without “Bioversal”, as well as at “Bioversal” concentrations of 6 and 100 mg/L were thereby added to the aqueous phase and their recovery and distribution was observed on three different segments (water surface, water column and vessel wall) in the waterbody for 72 hours (for details on the methodology see FRITZ 2001 b).

In comparison to a check test (diesel without “Bioversal” additive), in which the main fractions of the mineral oil residues used were primarily recovered at the surface of the water and at the vessel wall as absorbing phase, it was shown that the distribution of diesel oil and lubricants in the presence of Bioversal generally reduced to the benefit of the water column (meaning the mineral oil fractions are dislodged from the water surface and from the vessel wall to the water column). On the other hand the impact of “Bioversal” with increased application times led to a general drop in the recovery rate of the mineral oil fractions used. Interpreting this finding is difficult, however two possible interpretations are suggested: Firstly, the temporal reduction of the recovery rate for the hydrocarbon fractions used in more or less all segments investigated could indicate that blend phases arise under the effect of “Bioversal”, which promote the volatility of the mineral oil residues in the air, so that this has something to do with essentially temporally reduced availabilities of the hydrocarbon residues in the water due to physical dislocation processes from the water in the air. Secondly it is also imaginable however that the mineral oil percentages used in the presence of “Bioversal” decrease due to the incipient microbial degradation processes, so that in this case a stimulating effect of “Bioversal” on microbial degradation processes must be considered. In fact, the test results were in any event already referred to in the first expert report (DALLINGER 2000), attesting to an apparent stimulating effect of “Bioversal” on the microbial degradation of added hydrocarbon residues.

Literature

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For the authenticity of the expert report:

A. Univ. Prof. Dr. Reinhard Dallinger (Expert)

APPENDIX: Table 1: Information on the toxicity test interpreted in the expert report for various organism groups with notes on the test authority, date, test material concentration, brief description of the test guidelines as well as any test results

Organism	Test Authority, Test Institute	Date	Test material (Concentration)	Test description (methodology)	Guidelines	Results
Bacteria:						
Vibrio fischeri	IFA Tulln	26/2/2001	Bioversal FW® in combination with 10 µL/L diesel*	Luminescent bacteria test (LumisTox, Dr. Lange) 30 min, 15°C	DIN 38412	NOEC for synergistic effect: 100 mg/L
			Bioversal QF® in combination with 10 µL/L diesel*			NOEC for synergistic effect: 100 mg/L
Algae:						
Selenastrum capricornutum	IFA Tulln	26/2/2001	Bioversal FW® in combination with 10 µL/L diesel*	Acute inhibiting effect of the cell proliferation, 72 hrs, 22°C	DIN 38412 L33 OECD 201	NOEC for synergistic effect: 24.6 mg/L
			Bioversal QF® in combination with 10 µL/L diesel*			NOEC for synergistic effect: 49.6 mg/L
Invertebrates:						
Daphnia magna	IFA Tulln	26/2/2001	Bioversal FW® in combination with 10 µL/L diesel*	Acute toxicity test, 48 hrs, 22°C	DIN 38412 L30 OECD 202	NOEC for synergistic effect: 100 mg/L
			Bioversal QF® in combination with 10 µL/L diesel*			NOEC for synergistic effect: 24.6 mg/L
Fish:						
Brachydanio rerio	Seibersdorf Research Centre (Toxicology)	January 2001	Bioversal FW® in combination with 10 µL/L diesel*	Acute toxicity test, 96 hrs, 21°C	EEC Commission Directive 92/96, C.1.	NOEC for synergistic effect: > 100 mg/L
			Bioversal QF® in combination with 10 µL/L diesel*			NOEC for synergistic effect: > 100 mg/L

* 10 µL/L diesel ("loading rate") correspond to a concentration von 8.3 mg/L