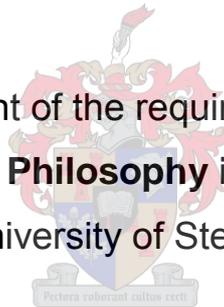

**Ecotoxicity and environmental fate of diesel and diesel blends produced
by Sasol's Fischer-Tropsch processes using natural gas and coal as
feedstock as well as biodiesel and biodiesel blends**

BY
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HONOUR - ACKNOWLEDGE - INSPIRE

This PhD is to HONOUR those who have paved the way for me in life, especially Franklin, Denise and Christopher; to ACKNOWLEDGE those who have walked with me, in particular Alétia; and to INSPIRE those who are still to walk their own paths through life, above all Ty.

DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously, in its entirety or in part, submitted it at any other university for a degree.

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Abstract

World crude oil demand and production is set to increase in the long term and is projected to increase from 82 barrels per day in 2007 to an estimated 104 million barrels per day in 2030 according to the International Energy Agency. The environmental challenges posed by the current and projected increased future fuel use, with specific reference to air, aquatic and terrestrial impact, are driving producers and legislators to change fuel specifications and consequently fuel properties to be less harmful to the environment. Traditionally transportation fuels are produced through crude oil refining but in South Africa more than one third of the liquid fuels are produced synthetically through catalytic conversion of gassified coal via the Fischer-Tropsch process by Sasol. Diesel from syncrude is referred to as synthetic diesel and the refiner must blend various hydrocarbon streams, effectively tailoring the diesel to its final composition. Biodiesel from renewable sources like vegetable oils is considered environmentally more acceptable than petrodiesel because of its high biodegradability in the environment, lower sulphur and aromatic hydrocarbon content as well as lowered particulate content in the exhaust emissions. The present research was aimed at evaluating whether the composition of diesels derived from different feed stocks, that included coal, natural gas, crude oil and soybean oil, would influence its biodegradability and ecotoxicity. Acute aquatic tests that included freshwater fish, crustaceans, algae and marine bacteria were used to determine the acute toxicity of diesels. In addition, quantitative structure activity relationship models were used to estimate the biodegradation and ecotoxicity properties of the diesels in an attempt to develop a cost effective tool to determine those properties. The results indicated that the 2-D GC technique quantitatively and qualitatively identified the hydrocarbon constituents in the diesels. The relevance of using the 2-D GC technique was in identifying and quantifying the hydrocarbon breakdown products and being used in a mass balance to confirm the potential biological breakdown processes of the materials used in the present study. The differences in theoretical oxygen demand (ThOD) of the different experimental diesel blends using various blending materials and biodiesel, emphasised and confirmed the importance of calculating the ThOD for the respective blending materials when measuring the biodegradation rates. Furthermore, the biodegradation hierarchy of Pitter and Chudoba (1990) in order of decreasing biodegradability: alkanes > branched alkanes > cyclo-alkanes > aromatic hydrocarbons, could be expanded to include FAME: FAME > alkanes > branched alkanes > cyclo-

alkanes > aromatic hydrocarbons. The biochemical pathways identified for the biodegradation of all the diesels was enzyme-enhanced β -oxidation. The present research also indicated that biodiesel addition to crude-derived diesels to increase the density to within the current required specifications for diesels cannot be a reality in SA because of the underdeveloped biodiesel industry. To increase the density by using biodiesel to within the specification for GTL diesel, more than 27% biodiesel would be required, which is currently is not achievable from an economic perspective as well as governmental national strategy perspective. The addition of biodiesel as lubricity enhancer seems more plausible, because less than 5% would be required for petrodiesels. The results on the ecotoxicity of the diesels and diesel blends demonstrated a general lack of acute toxic effect, especially for the fish and crustaceans used during the present study. Although algal and bacterial tests showed an effect at most of the WAF loading rates, none were high enough to enable the calculation of a median effect loading rate (EL_{50}). QSAR's, like EPI Suite, together with prediction models, like the Fisk Ecotoxicity Estimation Model, can be used to screen for ecotoxicity and biodegradability of hydrocarbons found in Petrodiesels. It was less applicable for the prediction of biodiesel constituents. The use of different cut-off values for the constituents of biodiesel could be developed in future research. The use of this combination enabled the present research into the potential toxicity of hydrocarbon mixtures to be conducted, especially since tests on individual constituents are impractical. QSAR's may provide a relatively cost-effective way to screen for potential environmental acceptability of such mixtures. The contributors to the toxicity of mixtures of hydrocarbons found in diesels were evaluated and it appears that paraffins contribute more to the overall toxicity than previously thought and aromatics less. By putting well-defined policies and incentives in place, a robust biodiesel industry could be created that will enable SA to contribute to the mitigation of the threat of climate change, to become less dependent on foreign oil and to develop rural agriculture. The key to energy security is not one solution to South Africa's energy needs, but a multifaceted approach to the complex subject of sustainable energy security. The end of the hydrocarbon era of energy is not in sight, at least for the near future, but soon even hydrocarbon energy in the form of coal and crude oil will have to be re-evaluated as SA's major energy resource for economic and energy security. In SA the potential of developing natural gas resources through fracking, nuclear, solar, wind, biological and even wastes to energy processes as well as better energy efficiency, in a balanced and diverse energy portfolio, could pave the way toward energy security in the long run.

Samevatting

Ru-olie aanvraag en produksie wêreldwyd is besig om toe te neem en die Internasionale Energie Agentskap projekteer dat wêreld ru-olie verbruik sal toeneem van 82 vate per dag in 2007 tot 'n beraamde 104 vate per dag in 2030. Die omgewings uitdagings wat huidige en toekomstige toename in brandstof verbruik, spesifiek die impak op lug gehalte, water- en grond, mag hê, is dryfvere vir produseerders en reguleerders om brandstof spesifikasies te verander om minder omgewings impak te veroorsaak. Brandstof vir vervoer doeleindes word oor die algemeen van ru-olie gemaak, maar in Suid Afrika word ongeveer 'n derde van die vloeibare brandstof gemaak deur middel van gekatilliseerde omskakeling van vergasde steenkool via die Fischer-Tropsch proses by Sasol. Diesel wat uit sintetiese ru-olie gemaak is, is sinteties en die raffineerder moet verskillende koolwaterstof strome meng om 'n finale produk te lewer. Biodiesel wat uit hernubare hulpbronne soos plant-olies en diervet gemaak word, kan oorweeg word vir die vervaardiging van meer omgewings aanvaarbare brandstof met laer swael en aromatiese koolwaterstof inhoud en ook minder partikel inhoud in die uitlaatgas. Die huidige navorsing het beoog om te evalueer of die samestelling van diesels wat vervaardig is uit verskillende hulpbronne, wat steenkool, aardgas, ru-olie en sojaboon olie ingesluit het, die biodegradeerbaarheid en ekotoksisiteit kan beïnvloed. Akute akwatiese toetse wat varswater vis, krustaseë, alge en marine bakterieë ingesluit het, was aangewend om die akute toksisiteit van die diesels te bepaal. Kwantitatiewe struktuur aktiwiteit verwantskaps modelle is ook gebruik om die biodegradeerbaarheid en ekotoksisiteits eienskappe van die diesels te beraam om vas te stel of 'n bekostigbare alternatief beskikbaar is om daardie eienskappe te bepaal. Die resultate het aangedui dat die 2D GC tegniek kwantitatief en kwalitatief gebruik kan word om die koolwaterstowwe in die diesels te identifiseer. Die benutting van die 2D GC tegnieke is egter om die koolwaterstof afbraak produkte te identifiseer en ook om die massa balans gedurende die biodegradering te bevestig. Die veskil in teoretiese suurstof aanvraag van die verskillende diesels het die belangrikheid daarvan blykbaar en bevestig om die teoretiese suurstof aanvraag korrek te bereken en sodoende die biodegradasie korrek te bepaal. Verder kan die biodegradasie hierargie van Pitter en Chudoba (1990) volgens afnemende biodegradasie: alkane > vertakte alkane > siklo-alkane > aromatiese koolwaterstowwe, uitgebrei word om vetsuur-metielesters in te sluit: vetsuur-metielesters > alkane > vertakte alkane > siklo-alkane > aromatiese koolwaterstowwe. Die biochemiese roetes wat geïdentifiseer is vir die biodegradasie van

die diesels, was ensiem-verbeterde β -oksidasie. Die huidige navorsing het ook aangedui dat biodiesel toevoeging tot ru-olie vervaardigde diesel om die digtheid te verhoog tot binne huidige spesifikasies is nog nie lewensvatbaar in Suid Afrika nie as gevolg van die onderontwikkelde biodiesel industrie. Om die digtheid te verhoog met biodiesel tot binne spesifikasie verg meer as 27% biodiesel en is huidiglik nie haalbaar vanuit 'n ekonomiese perspektief en ook nie vanuit 'n regerings nasionale strategie perspektief nie. Die toevoeging van biodiesel as lubrisiteits vervetering blyk meer van toepassing te wees aangesien minder as 5% biodiesel toevoeging benodig sou wees. Die resultate van die ekotoksiseits toetse het 'n algemene gebrek aan akute toksiseits effek aangedui, veral vir vis en skaaldiere wat in die huidige studie gebruik is. Howel alge en bakteriële toetse daarop gedui het dat 'n toksiese effek wel aanwesig was, was dit gering en kon die median effektiewe ladings koers (EL_{50}) nie bepaal word nie. QSARs, soos Epi Suite, tesame met voorspellings modelle, soos die Fisk Ecotoxicity Estimation Model, kan gebruik word om ekotoksiseit en biodegradeerbaarheid van koolwaterstowwe in petrodiesels te beraam, alhoewel dit minder van toepassing was op biodiesel. Die gebruik van ander afsny waardes spesifiek vir biodiesel kan oorweeg word in toekomstige navorsing. Die molekules wat bygedra het tot die toksiseit van die koolwaterstof mengsels was geëvalueer daar is gevind dat die paraffiniese molekules meer begedra het tot die totale toksiseit en die aromate minder. Deur goed gedefinieerde beleid en aansporings meganismes inplek te sit, kan 'n biodiesel industrie in SA geskep word wat SA sal help om by te dra tot die bekamping van klimaats vordering en sodoende minder afhanklik te wees van buitelandse olie en ook landbou in SA te bevorder. Die sluetel tot energie sekuriteit is nie een oplossing vir SA se energie aanvraag nie, maar eerder 'n veelsydige benadering tot die komplekse onderwerp van volhoubare energie sekuriteit. Die einde van koolwaterstof energie is nog nie in sig nie, ten miste nie in die nabye toekoms nie, maar binnekort sal selfs koolwaterstof energie in die vorm van steenkool en ru-olie heroorweeg moet word as SA se hoof energie hulpbronne vir ekonomiese en energie sekuriteit. In SA moet die potensiaal van natuurlike gas ontginning deur middel van hidrauliese breking, kernkrag, wind energie, biologiese energie en selfs afval tot energie prosesse bestudeer word, so-ook beter energie doeltreffendheid om sodoende 'n gebalansweerde energie portefuelje te skep wat die weg sal baan na energie sekuriteit op die lang termyn.

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Abbreviations

2-D	Two Dimensional
BOD	Biochemical Oxygen Demand
BTT	Biofuels Task Team
C	Carbon
CES	Coordinated Environmental Services
Ca	Calcium
CAS	Chemical Abstract Service
CFB	Circulating Fluidised Bed
CIE	Compression Ignition Engine
Cl	Chlorine
CO	Carbon Monoxide
CO ₂	Carbon Dioxide
CONCAWE	Conservation of Clean Air and Water in Europe
CTL	Coal-To-Liquids
DBM	Diesel Blending Material
DHT	Distillate Hydro-Treater
DME	Department of Minerals and Energy
DSC	Distillate Selective Cracker
EC ₅₀	Median Effect Concentration
EEC	European Economic Community
EL ₅₀	Median Effect Limit
EPI Suite	Estimation Program Interface Suite
ERI	Energy Research Institute
EU	European Union
ESKOM	Electricity Supply Commission
FAME	Fatty Acid Methyl Ester
FEEM	Fisk Ecotoxicity Estimation Model
FFS	Fuel Filling Stations
FID	Flame Ionisation Detector
F-T	Fischer-Tropsch
GC	Gas Chromatography

GE	General Electric
GTL	Gas-To-Liquids
H ₂	Hydrogen
HC	Hydrocarbon
HT-FT	High Fischer-Tropsch
IB	Inherently Biodegradable, fulfilling the criteria
IBN	Inherently Biodegradable, Not fulfilling the criteria
IEA	International Energy Agency
ISO	International Organization for Standardization
K _{OW}	Octanol-Water Coefficient
LC ₅₀	Median Lethal Concentration
LT-FT	Temperature Fischer-Tropsch
Mg	Magnesium
N	Nitrogen
Na	Sodium
Natref	National Refiners
O ₂	Oxygen
OECD	Organisation for Economic Cooperation and Development
OFID	OPEC Fund for International Development
OPEC	Organisation of the Petroleum Exporting Countries
P	Phosphorus
QSAR	Quantitative Structure Activity Relationship
R&D	Research and Development
RB	Readily Biodegradable
RBN	Readily Biodegradable, Not meeting 10-day window criteria
S	Sulphur
SA	South Africa
SANS	South African National Standard
SAR	Structure Activity relationship
Sasol	South African Synthetic Oil
SPD	Slurry Phase Distillate
STP	Standard Temperature and Pressure
ThOD	Theoretical Oxygen Demand
TOF-MS	Time Of Flight – Mass Spectrophotometry

TUa	Acute Toxicity Unit
UCT	University of Cape Town
US EPA	United States Environmental Protection Agency
USA	United States of America
VOC	Volatile Organic Compound
WAF	Water Accommodated Fraction
WWTW	Wastewater Treatment Works

INTRODUCTION

World crude oil demand, although currently in decline due to the global economic slowdown, together with production is set to increase in the long term. The production of world crude oil is projected to increase from 82 barrels per day in 2007 to an estimated 104 million barrels per day in 2030 according to the International Energy Agency's World Energy outlook 2008 (OECD/IEA, 2008). The environmental challenges posed by the current and projected increased future fuel use, with specific reference to air, aquatic and terrestrial impact, are driving producers and legislators to change fuel specifications and consequently fuel properties to be less harmful to the environment. Gasoline, diesel, aviation gas and jet fuel are the main energy sources for transport in South Africa with small quantities of coal and electricity also being utilised. Road vehicles dominate transport energy usage in South Africa. The large quantities of transport fuel and other petroleum products have the potential to cause significant environmental pollution especially through spillages, leakages from storage facilities and illegal dumping which contaminate soil and natural water resources. Although the changes in the fuel quality can result in less environmental impact by end-users, it might result in refineries investing in technologies and processes that are more energy intensive than current practices or utilizes substances that more hazardous substances than those currently in use (De Klerk, 2007a).

There are many life-cycle stages of fuel which may impact on the environment and it should be noted that this study focused mainly on diesel as a product and not per se on the combusted result. In the past the focus on air emissions was the main driver for changes in fuel specification with reduced levels of benzene in gasoline and sulphur in diesel as recent examples of such fuel specification changes. In South Africa (SA) where fuel filling station (FFS) attendants are responsible for the servicing of customers' vehicles, the health risks associated with prolonged exposure to potential volatile organic compounds (VOCs) from the fuels are the possible reason for the strong focus on human health regarding fuel constituents. This emphasis on human health has shifted to a more balanced approach with regard to biological effects of transportation fuel on humans and wildlife in recent years.

Traditionally transportation fuels are produced through crude oil refining and from a global perspective this will remain the case for many years to come, but in SA more than one third of the liquid fuels are produced synthetically through catalytic conversion of gassified coal via the Fischer-Tropsch process (Sasol Facts, 2008) by Sasol. The remaining two-thirds are supplied via refineries that import crude oil. In addition to this, PetroSA is able to produce small quantities from natural gas reserves off the south eastern coast of SA. According to a report from the Department of Minerals and Energy – Eskom – Energy Research Institute, UCT (DME-ESKOM-ERI, UCT, 2002), SA will continue to import crude oil for refineries and Sasol will continue to play an important role in providing the country with synthetic fuel. Diesel is one of the fuels produced from various feedstocks including crude oil, coal (Coal To Liquids or CTL) and natural gas (Gas-To-Liquid or GTL) via the Fischer-Tropsch (F-T) process. There is also a drive from Sasol to support biodiesel produced from renewable sources such as vegetable oils derived from plants. The most notable effort being the agreement between Sasol, the Central Energy Fund and Siyanda Biodiesel together with Lurgi AG as technology partner for a potential biodiesel plant that could produce 100 000 tons of soya bean based biodiesel (Sasol-CEF-Siyanda Biodiesel media release, 2006).

Sasol is currently producing significantly more petrol than diesel at its Secunda plants because the current demand for petrol is higher compared to diesel (DME-ESKOM-ERI, UCT, 2002). However with diesel vehicles becoming more efficient and convenient to use together with the increased use of diesel vehicles in land transportation, the demand for eco-friendly diesel is set to increase in coming years as was the case in Europe (DME-ESKOM-ERI, UCT, 2002) since diesel is showing improved performance and fuel economy due to the application of modern technologies such as fuel injection and turbo charging in engines.

Synthetic Fuel: Coal To Liquids (CTL) and Gas To Liquids (GTL)

The Fischer-Tropsch process produces synthetic crude (syncrude) that is a mixture of hydrocarbons and is comparable to crude oil but with several major differences (Table 1). Sasol syncrude is classified as either high temperature Fischer-Tropsch (HT-FT) or low temperature Fischer-Tropsch (LT-FT) depending on the process and catalyst being used which results in syncrude with different properties. The paraffin fraction, also referred to as kerosene, of conventional crude oil is said to pose the greatest pollution problem

(Solano-Serena *et al.*, 2000), but can also lead to opportunities in remediation since the molecular structure, number of carbon atoms and the heterogenous atoms all influence both the toxicity and degradability of the products refined from the crude oil. It is specifically noted that saturated molecules in crude oil like paraffins of intermediate carbon length ($C_{10} - C_{20}$) biodegrade more readily than other organic molecules (Subarna *et al.*, 2002).

Table 1: Comparison of the major differences between syncrude and crude oil (De Klerk, 2007a).

Property	HT-FT	LT-FT	Crude oil
Paraffins	> 10%	Major product	Major product
Naphthenes	< 1%	< 1%	Major product
Olefins	Major product	> 10%	None
Aromatics	5 – 10%	< 1%	Major product
Oxygenates	5 – 15%	5 – 15%	< 1% O (heavy)
Sulphur species	None	None	0.1 – 5% S
Nitrogen species	None	None	< 1% N
Organometallics	Carboxylates	Carboxylates	Phorphyrines
Water	Major by-product	Major by-product	0 – 2%

Examples of processes that produce the HT-FT syncrude are the Sasol Advanced Synthol in Secunda (SA) and the Synthol circulating fluidised bed (CFB) in Mosselbay (SA). Examples of processes that produce the LT-FT syncrude are the Shell Middle Distillate Synthesis in Bintulu (Malaysia), the Sasol Slurry Phase Distillate process used in Qatar and Sasolburg (SA) and also the Arge process used in Sasolburg (De Klerk, 2007a).

The liquid fuels production utilising the F-T process consists of three main steps or processes after obtaining a good feedstock. An important factor in obtaining a good carbonaceous feedstock for the F-T process is that it must contain carbon and also sufficient hydrogen, because hydrogen increases the efficiency of the hydrocarbon conversion (Steynberg & Dry, 2004). Coal, biomass, or natural gas are good feedstocks for hydrocarbon conversion through the F-T process of which coal is an important hydrogen lean feedstock.

The first process is the production of synthesis gas (syngas) from coal or biomass which is called gassification, where coal, usually of lower quality than that used in power stations,

steam and oxygen are passed over coke at high temperatures and pressures to produce hydrogen and carbon monoxide (Steynberg & Dry, 2004). Two gasification processes exist viz. high temperature and low temperature gasification. High temperature gasification is used by companies like Shell and General Electric, however, Sasol uses low temperature gasification through the Lurgi dry-ash coal gasification technology (De Klerk, 2007b). The products of gasification are mainly carbon monoxide (CO), hydrogen (H₂) and carbon dioxide (CO₂). The low temperature coal gasifiers also produce gas liquor, tar oils and condensed tar and are called pyrolysis products which are separated from the syngas and refined in a tar refinery (Leckel, 2006). These products are high in aromatic compounds that contain heteroatoms like sulphur, nitrogen and oxygen. The tar acids are rich in phenols, cresols, xlenols and naphthols. Aromatic compounds have a high octane number and should be retained for the naphtha and petrol fractions (Leckel, 2006). Aromatics are added to diesel to increase the density to meet the required specification; however the cetane number is lowered in the process. It is clear that the tar refinery plays an integral role in fuel processing opportunities downstream from the F-T process.

When using natural gas as feedstock, gasification is not required. Instead the natural gas, mainly methane (70% - 90%) and ethane (5% - 15%), is reformed by a process called methane reforming to produce syngas that contains significantly less CO₂ in comparison to what is produced through gasification of coal, which makes it more attractive from an environmental point of view.

The second step is the F-T process and can be either high temperature F-T (HT-FT) or low temperature F-T (LT-FT). The desired products and the feedstock used are the most important factor in F-T synthesis. The catalyst also plays an important role. An iron catalyst is preferred when the feedstock is coal as cobalt is more expensive and when coal is used catalyst poisoning is difficult to prevent (Steynberg & Dry, 2004). The product from the F-T process, whether LT-FT or HT-FT, is called synthetic crude (syncrude).

The third step in the application of the F-T process is product upgrading, commonly referred to as refining. This step is wholly dependent on the desired products. In a coal fed process as is used in Secunda, there are four main refineries; one for each of the condensates, the oil, the reaction water and the tar. Together the products from the various refineries are blended and formulated to create the products that go into vehicles

at the garage pump stations. Figure 1 gives an illustration of the basic processes used with the F-T technology (De Klerk, 2007b).

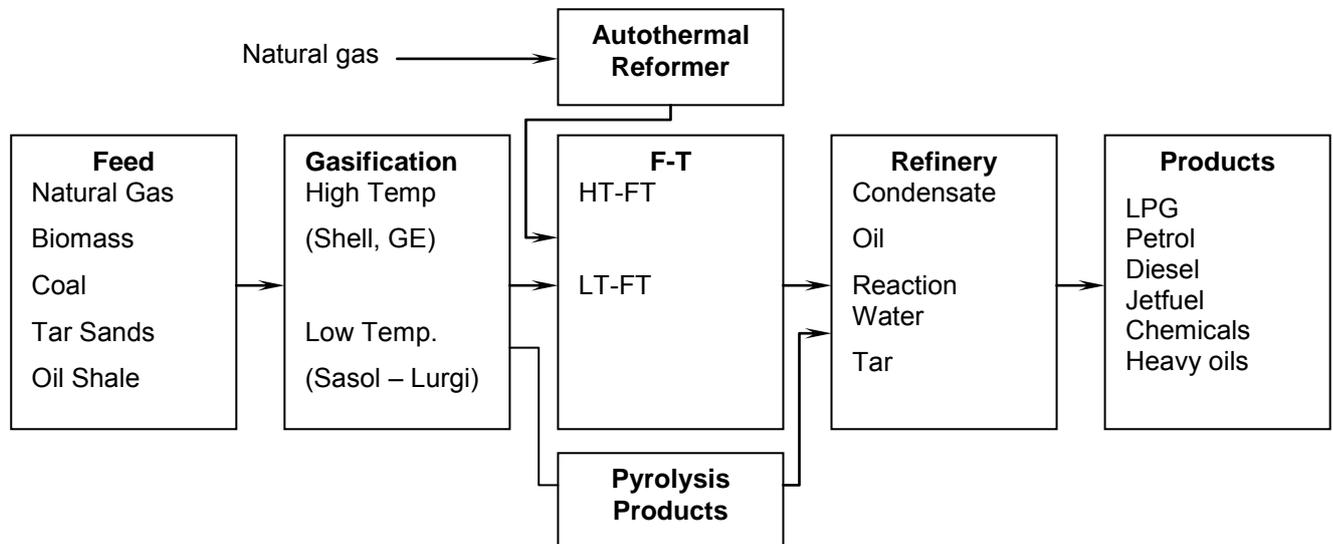


Figure 1: A simple layout of the basic steps or processes required to produce liquid fuels, fine chemicals and other downstream products when utilising the Fischer-Tropsch technology. From De Klerk (2007b).

In SA Sasol has been operating since 1955 and at present produces 150 000 barrels per day of synthetic fuel (gasoline, diesel and kerosene) through its HT-FT process (Schaberg *et al.* 1999). The LT-FT process traditionally employed the Arge technology for wax manufacture; however a new technology, the Sasol Slurry Phase Distillate Process (SPD®), has been developed and is currently in full-scale operation in the Oryx Facility in Qatar to produce speciality waxes and paraffins from syngas (Schaberg *et al.* 1999). The syncrude from both HT-FT and LT-FT requires additional refining into the various fuel products that Sasol produces with the added advantage that the product work-up is less severe than it is in a crude refinery due to the low metal content of the syncrude and also less complex as a result of a less complex syncrude slate.

Diesel properties

Crude oil derived diesel (Petrodiesel) is a complex mixture of petroleum hydrocarbons composed mainly of paraffin, naphthenic and aromatic compounds. Conventionally diesel is refined from crude oil which is a complex series of interdependent processes that

converts the crude oil into high-value end-products through separation (distillation), upgrading (hydrotreating) and conversion (catalytic cracking and hydrocracking) (Chevron Products Company, 1998). The cost of refining is mainly affected by the quality of the crude oil where low-gravity oils (also called thick crude oils) are more energy intensive to refine than high-gravity crude oils (also called thin crude oils). The refiner of crude oils has limited control over the final composition and properties of the final blend of diesel, which is mainly dictated by the crude oil feed. Refining high-value end-products, including diesel, from syncrude, as stated earlier, is less complex and subsequently less costly. Diesel from syncrude is referred to as synthetic diesel. To meet the required diesel specification, the refiner must blend various hydrocarbon streams, effectively tailoring the diesel to its final composition.

The desired products from the refinery are guided by the fuel specification and not necessarily what is good for the environment. Refineries need to change their processes to accommodate the latest fuel specification and a good South African example is the change from leaded fuel to unleaded and lead replacement fuel in 2005 and 2006. In recent times the trend has been to reduce sulphur in diesel fuels to less or equal to $10\mu\text{g.g}^{-1}$ and benzene reduction in petrol to less or equal to 1% (vol basis) (De Klerk, 2007a). Global trends see Petrol octane requirements increasing and the composition is becoming more paraffinic. Diesel cetane index is increasing to greater or equal to 45 and consequently the density range is narrowing and the use of heavy fuel fractions are declining.

The current diesel specifications adopted by Sasol are presented in Table 2. There is a difference in the current South African diesel specification (SANS 342:2005, 2005) compared to the Sasol specification since the Sasol production diesels are blended according to Sasol internal adopted specifications often comparable to international diesel specifications. The Sasol production diesel is superior in quality to diesels complying with the minimum SANS 342:2005 (2005) specification. The most important properties of the fuel that impacts on the environment are the sulphur content, aromatic and poly-aromatic content and olefin content.

Table 2: Diesel specification of crude derived, coal derived syncrude (CTL), natural gas derived syncrude (GTL) and soybean derived biodiesel.

Property	Current SA Requirement *		Sasol diesel	
	Standard Grade	Low Sulphur Grade	CTL Diesel #	GTL Diesel ##
Distillation temp. for 95% (by volume) recovery [°C], max	362	362	350	360
Flashpoint [°C], min	55	55	>60	60
Sulphur content [%], max	0.3	0.05	<0.0005	<0.0001
Cetane Number, min	45	45	>70	<72
Copper strip corrosion (3h at 100°C), max	1	1	-	-
Cold filter plugging point (CFPP) [°C], max	-4 or 3	-4 or 3	-14	-7
Carbon residue on 10% (by volume) distillation residue [%] (by mass), max	0.2	0.2	-	-
Ash content [%], max	0.01	0.01	-	-
Water content (by volume) [%], max	0.05	0.05	<0.015	0.020
Total contamination [mg/kg], max	50	50	-	-
Lubricity, corrected wear scar diameter [wsd 1.4] at 60°C, max	-	460	400	-
Viscosity at 40°C [mm ² /s]	2.2 to 5.3	2.2 to 5.3	>1.5	2.55
Density at 20°C [kg/L], min	0.800	0.800		0.770
Oxidation stability [mg/100mL], max	2.0	2.0	>2	1.4
Aromatic Content (by weight) [%], max	-	-	<0.5	0
Poly-aromatic Content (by weight) [%], max	-	-	0	0

* Detailed requirements for SANS 342:2005 certification (SANS 342, 2005)

CTL Requirement for SPD™ LTFT (Maree, 2007)

HRCU SPD™ LTFT Diesel quality (Maree, 2007)

The specifications have a direct effect on the performance of the fuel in terms of its combustion in the engine, the emissions it produces, its persistence in the environment and its toxicity to humans and wildlife. Diesels with high aromatic concentrations have poor self-ignition qualities and have a tendency to produce more soot on combustion which is the major contributor to smoke and particulate matter formation (Maree, 2007). In addition aromatics lower the cetane number which results in poor cold-starting, increased combustion noise and increase nitrogen oxide and hydrocarbon emissions. Diesel derived from crude oil contains aromatics and the level of refining required to remove all aromatics is not economically viable. Consequently it is allowed to form part of the mixtures and has an effect on the density specification of diesel since paraffin, iso-paraffin and cyclo-paraffin

have a much lower hydrogen to carbon (H/C) ratio than aromatics which results in a lower density. In order to increase the density of Sasol's diesel to the correct specification, the addition of limited amounts of aromatics is allowed.

Biodiesel

Biodiesel is defined as monoalkyl esters derived from vegetable oils and animal fats and also from oil-producing algae (Demirbas, 2007). Biodiesel is largely suitable for use as an alternative to petrodiesel because its physical properties are close to that of petrodiesel; it could be economically competitive; it can be transported and stored in existing infrastructure; and can be used in conventional compression ignition engines (CIE) with little or no modification required. In addition biodiesel is considered a renewable energy source that is environmentally more acceptable than petrodiesel because of its high biodegradability in the environment, lower sulphur and aromatic hydrocarbon content and particulate content in the exhaust emissions (Demirbas, 2007). The environmental aspects of biodiesel will be further discussed in the chapters that follow. The world leaders in biodiesel production in 2005 was headed by Germany (1 919ML/annum) from Rapeseed, followed by France (511ML/annum) from Soybean and the USA (291ML/annum) from Rapeseed (Escobar *et al.* 2009).

The interest in fuels produced from biomass is not a novel concept and engine builders in the late 1800's and early 1900's like Rudolph Diesel, who developed the first diesel engine, experimented with vegetable oil to power their engine technologies. The biofuel industry in those days was not viable compared to the relatively cheap, easily attainable and wide offering of different petroleum derived fuels (Agarwal, 2007; Murugesan *et al.*, 2009; Escobar *et al.*, 2009). Not much has changed since then and petroleum derived vehicular fuels still make up the biggest portion of transportation fuel in South Africa and globally where fossil fuels account for more than 57.7% of the global transportation fuels (IEA, 2008). Globally, biofuels have attracted serious attention since the turn of the millennium with the previous waves of increased research into biodiesel occurring in the 1940's and late 1970's and early 1980's (Balat & Balat, 2008). The renewed interest in biofuels is related to an increased global energy demand in fossil fuel production and use and with it the environmental concerns as a result of potential atmospheric, terrestrial and aquatic pollution.

Biofuel is not widely used in South Africa and apart from a small number of farmers who produce their own biodiesel and bio-ethanol for personal use on a relatively small scale, no large commercial scale production facilities exist. The SA government realised that the country's natural energy resources should be fully utilised according to its White Paper on Energy Policy (DME, 1998) and subsequently developed a supporting document called the White Paper on Renewable Energy which recognises that the medium- and long-term potential of renewable energy is significant (DME, 2003). In this White Paper the goal of 10 000 GWha renewable energy contribution to final energy consumption by 2013 was set. In 2006 a Biofuels Task Team (BTT) was established to develop an industry strategy to achieve the 2013 goal. In 2006 a draft Biofuels Industry Strategy was approved by Cabinet for public consultation where 4.5% biofuels penetration was projected based on the country's climatic conditions, land availability, agricultural potential and food security and various other socio-economic considerations (DME, 2007). After public consultation the Biofuels Industry Strategy adopted a 2% biofuels penetration, amounting to approximately 400 million litres per annum, by 2013. Production crops that were identified were sugar cane and sugar beet for bio-ethanol production and sunflower, canola and soya bean oils for biodiesel production. Maize and *Jatropha* were excluded based on food security concerns and environmental concerns respectively (DME, 2007).

The South African Biofuels Industry Strategy target of 2% biofuels penetration will affect Sasol's fuel pool in potentially two ways. The first could be to blend bio-ethanol into gasoline and/or blend biodiesel into the diesel pool. Sasol Technology's Fuel Technology research department investigated several options and preliminarily opted to further research into the quality of biodiesel from soya bean scenario. Because other sources such as Palm oil (Escobar *et al.*, 2009) could potentially yield more oil per land area compared to soya bean oil and the potential of micro-algal oil (Demirbas, 2008) yielding potentially the most oil compared to terrestrial plants, Fuels Technology decided to invest some time and resources into evaluating those oil sources for biodiesel production and the quality of the biodiesel. According to the South African national standards (SANS 342, 2005), up to 5% biodiesel, related to volume, is allowed to be blended into conventional automotive diesel. When using biodiesel as blending agent, the biodiesel should comply with the South African national standards (SANS 1935, 2005) which is detailed in Table 3.

Table 3: Detailed South African national standards requirements for automotive biodiesel fuel.

Property	Requirements	Test Method
Ester content [% mass fraction], min	96.5	EN14103
Density at 15°C [kg/m ³]	860 - 900	ISO 3675, ISO 12185
Kinematic viscosity at 40°C [mm ² /s]	3.5	ISO 3104
Flash Point [°C], min	120	ISO 3679
Sulphur content [mg/kg], max	10.0	ISO 20846, ISO 20884
Carbon residue (on 10% distillation residue) [% mass fraction], max	0.3	ISO 10370
Cetane number, min	51	ISO 5165
Sulfated ash content [% mass fraction], max	0.02	ISO 3987
Water content [% mass fraction], max	0.05	ISO 12937
Total contamination [mg/kg], max	24	EN 12662
Copper strip corrosion (3h at 50°C) [Rating], max	Class 1	ISO 2160
Oxidation stability (at 110°C) [h], min	6	EN 14112
Acid value [mg KOH/g], max	0.5	EN 14104
Iodine value [g of iodine/100g of FAME], max	140	EN 14111
Linolenic acid methyl ester [% mass fraction], max	12	EN 14103
Polyunsaturated (≥ 4 double bonds) methyl esters [% mass fraction], max	1	-
Methanol content [% mass fraction], max	0.2	EN 14110
Monoglyceride content [% mass fraction], max	0.8	EN 14105
Diglyceride content [% mass fraction], max	0.2	EN 14105
Triglyceride content [% mass fraction], max	0.2	EN 14105
Free glycerol content [% mass fraction], max	0.02	EN 14105, EN 14106
Total glycerol content [% mass fraction], max	0.25	EN 14105
Group I metals (total of Na and K) [mg/kg], max	5.0	EN14108, EN 14109
Group II metals (total of Ca and Mg) [mg/kg], max	5.0	prEN 14538
Phosphorus content [mg/kg], max	10.0	EN 14107
Cold Filter Plugging Point (CFPP)		
Winter [°C]	-4	EN 116
Summer [°C]	+3	

Biodiesel properties differ with the plant oil it is produced from; however the difference is not great and close to that of petrodiesel. This is one of the reasons that make biodiesel compatible with modern engine technologies and also a highly acceptable alternative biofuel to replace petrodiesel with. The major advantages (Demirbas, 2007; Mudge & Pereira, 1999; Knothe et al., 2005; Knothe et al., 2006; Ma & Hanna, 1999; Mittelbach &

Remschmidt, 2004; Speidel et al., 2000; Zhang et al., 2003) and disadvantages (Demirbas, 2007; Demirbas, 2006; EPA, 2002; Prakash, 1998) of biodiesel are presented in Table 4.

Table 4: Major advantages and disadvantages of using biodiesel in compression ignition engines (Demirbas, 2006; Demirbas, 2007; EPA, 2002; Knothe et al., 2005; Knothe et al., 2006; Ma & Hanna, 1999; Mittelbach & Remschmidt, 2004; Mudge & Pereira, 1999; Prakash, 1998; Speidel et al., 2000; Zhang et al., 2003).

Advantages	Disadvantages
It comes in a liquid phase that is portable	Higher viscosity
Readily available	Lower energy content
It is renewable	Higher cloud point and pour point
High combustion efficiency	Higher nitrogen (NO _x) emissions
Very low sulphur and aromatic content	Lower engine speed and power
High Cetane number	Injector coking
High biodegradability rate	Engine compatibility
Produced locally (domestic origin)	High price
High Flash Point	Higher engine wear
Inherent lubricity properties	Cold-start problems

Although the disadvantages of biodiesel present several technical challenges in terms of its use in the internal combustion engine, its production from potential food sources could result in it competing with food resources, especially agricultural land and water. While biofuel can contribute to climate change mitigation and rural agricultural development, accelerated growth of first-generation biofuels production was found to be threatening the availability of adequate food supplies for humans (OFID, 2009a) where biofuels development scenarios indicated a strong relationship between agricultural prices and the share of first-generation biofuels in total transport fuels. The OFID (2009b) report also found that the “green” contribution of first-generation biofuels is seen as deceptive and that the second-generation biofuels appeared to offer more interesting prospects in terms of sustainability.

The risk of increased food insecurity that may increase the number of people at risk of hunger by more than 15% in the developing countries needs to be considered when interpreting an enhancement of “energy security” by achieving a share of biofuels in transport fuel of just 8% in the developed countries. The international community needs to

view food security and fuel security as interdependent and requiring integrated solutions since both are critical to human survival and well-being. Biodiesel is considered one of the alternatives for petroleum diesel if it can be produced in sufficient volumes at economically sustainable costs and does not interfere with the food security of a country, which means it will probably have to be made from a non-food source. The main variables that influence the economic feasibility of biofuels and in particular biodiesel are plant capacity (tons/ha), price of feedstock, ratio of biodiesel to byproduct (You *et al.* 2008). Several studies on the economic evaluation of biodiesel production have indicated that it costs more (up to 1.5 times more than petroleum diesel) to produce than petroleum diesel on a commercial scale (You *et al.* 2008; SAC, 2005; DEFRA, 2003; Bender, 1999).

Quantitative Structure Activity Relationship models

A quantitative structure activity relationship (QSAR) analysis is the mathematical relationship between biological activity of a compound and computed (or measured) properties that depend on the molecular structure. By using measured data of well studied compounds, the physical-chemical as well as biological activity of a new formulation with a chemical structure closely related to the known compound can be estimated or predicted mathematically. QSAR models can be used where experimental data are absent and reliable QSAR models have been developed for narcotic substances such as hydrocarbons, alcohols, ketones, and other aliphatic chlorinated hydrocarbons. Several case studies (Votta & White, 2000; Weeks *et al.*, 2002; Bayer Corporation, 2003; Eaton Aeroquip Inc., 2004; Chun *et al.*, 2002) indicated that the use of QSAR derived data can be accurate and cost saving and most importantly can be used as an “early warning” tool where exposure to the environment and to workers can be identified and quantified mathematically. A high level of accuracy was achieved in the PPG Industries study where 38 compounds were evaluated using structure activity relationship (SAR) and compared with measured acute aquatic toxicity data and a 91% agreement was achieved (Chun *et al.*, 2002).

The need for estimation methods to estimate physical and chemical properties as well as biological response was realised during the mid to late 1980's when standardised test procedures such as the Organisation for Economic Co-operation and development (OECD) became well established and the generation of physical-chemical as well as toxicity and ecotoxicity data became standard practise. The progress in the development

of computer hardware also made the move to modelling software a reality. Because any chemical substance can be defined by its physical-chemical properties, its toxicity and ecotoxicity to humans and wildlife was not used as frequently. Since all substances, natural or anthropogenic, can potentially be threatening to the environment, the importance of knowing its toxicological properties is very important. The process of determining a substance's toxicological properties can be costly and when toxicity and/or ecotoxicity assessments are performed, it usually involves sentinel organisms. If one then considers the amount of chemicals that are developed and registered every year, it is not difficult to understand the need for methods that could reduce cost as well as reduce the need to use sentinel species. It is also interesting to note that throughout the development of a single product that eventually reaches the consumer; several other formulations were tested and evaluated for both its efficiency for its use and whether it conforms to its regulatory compliance.

Estimation models have been in use over the last two decades and are becoming more important and reliable with the development of the technology. The main concern with the use of estimation models is whether their accuracy, which can vary between 20% and 50% uncertainty, is sufficient to be used from an environmental perspective. Jørgensen *et al.* (1998) are of the opinion that it is sufficient based on the deduction that it is the purpose of the research and development of chemicals to ensure that the substances making it into registration, production and eventually use, pose very low or no risk to humans or the environment. It is thus important that appropriate safety factors are used to compensate for complexity of the media, the presence of chemical mixtures, and the limitations of the models themselves. It is currently an almost impossible task to assess all substances for the ten minimum properties that the OECD recommends should be known about a substance and it is believed that we have determined less than 1% of the properties by measurement for most of the chemicals in use today.

The OECD embarked on the (Q)SAR Project from 1991 to 1993 where they compared QSAR estimation with a base-set test data of 175 chemicals which was published jointly by the US EPA and the OECD (OECD, 1994). During the mid 1990's several additional OECD initiatives were undertaken to assess variety of QSAR methodologies. Today many QSAR models exist for many different uses and estimations and it is advisable to use as many different QSAR models as possible to obtain the most accurate range for a particular biological parameter that is estimated. This can be costly as the estimation software

programs can be expensive. Comparisons of various estimation models, including their associated methodologies and regulatory application, have been extensively reviewed by Tunkel *et al.* (2005), Jawarska *et al.* (2003), Cronin *et al.* (2003a), Cronin *et al.* (2003b), Moore *et al.* (2003), Perkins *et al.* (2003), Walker *et al.* (2003), Jawarska *et al.* (2002), Tunkel, *et al.* (2000) and Rorije *et al.* (1999).

EPI Suite™ is a freely available QSAR software program and can be downloaded from the US EPA website. It consists of thirteen individual models that can estimate physico-chemical properties, ecotoxicological properties, biodegradability and fate of organic substances. It uses a databank of information with experimental values from substances with chemical abstracts service (CAS) numbers. The Chemical Abstracts Service is a division of the American Chemical Society that assigns numerical identifiers to all chemicals described in literature. On 22 June 2011 at 15:24 Central African Time the CAS registry contained 62 909 950 organic and inorganic substances and approximately an additional 50 000 substances are added weekly (CAS registry, 2011). It is important to note that EPI Suite™ is a screening-level predictive tool and has strengths, weaknesses and limitations. This tool is specifically intended and developed to quickly screen substances for release and exposure potential. The estimations resulting from EPI Suite™ should not replace experimental data.

Research Objectives

This research aimed to evaluate whether the composition of diesels derived from different feed stocks, that included CTL, GTL, crude oil and soybean oil, would influence its biodegradability and ecotoxicity. The effect of blending biodiesel (derived from soybean oil) with GTL- and crude-oil derived diesel on biodegradation and ecotoxicity was also investigated. This study also assessed the applicability and accuracy of QSAR derived ecotoxicity and biodegradability estimations for the diesels and diesel blends that were tested.

The hypotheses were:

- The biodegradation rate is influenced by the molecular composition of the diesels derived from crude, GTL, CTL and soybean oil.

- The toxicity is influenced by the molecular composition of the diesels derived from crude, GTL, CTL and soyabean oil.
- Biodiesel addition to diesel derived from crude and GTL influences biodegradability and toxicity of such diesel blends
- QSAR software estimated biodegradation rate and toxicity of diesels and diesel blends accurately.

-2-

Materials & Methods

2.1. Fuels

The diesels and diesel blending materials were received from the Sasol Technology, Research and Development, Fuels Research. The complete diesels were used as they were received and kept in a refrigerator at 4 °C. The fuels originated from Coal-to-Liquids (CTL) facilities, Gas-to-Liquids (GTL) facilities, Crude oil refineries, European fuel manufacturers and Biodiesel manufactures. The materials tested included the following unadditised diesel products:

Complete diesels

- Sasol GTL 100% diesel
- Sasol Natref Final Diesel 100%
- EN590 diesel (diesel that meet physical properties of automotive diesel fuel sold in the European Union)
- Biodiesel 100 (Soybean derived fatty acid methyl esters (FAME)) (B100)
- CTL Distillate Hydrotreater (DHT) diesel
- CTL Distillate Selective Cracker (DSC) diesel
- CTL Creosote diesel blending material
- CTL CatPoly diesel blending material

Experimental diesels

The experimental diesels were prepared using the blending materials provided and that are used to prepare the diesel formulations for use in motor vehicles. The diesel blending material fractions were based on volume and not mass.

- 5% Biodiesel addition to EN590 diesel
- 10% Biodiesel addition to EN590 diesel
- 30% Biodiesel addition to EN590 diesel

- 0.1% B100 addition to GTL diesel (B0.1)

-
- 0.2% B100 addition to GTL diesel (B0.2)
 - 0.4% B100 addition to GTL diesel (B0.4)
 - 0.8% B100 addition to GTL diesel (B0.8)
 - 1.6% B100 addition to GTL diesel (B1.6)
 - 3.2% B100 addition to GTL diesel (B3.2)
 - 6.4% B100 addition to GTL diesel (B6.4)
 - 12.5% B100 addition to GTL diesel (B12.5)
 - 25% B100 addition to GTL diesel (B25)
 - 50% B100 addition to GTL diesel (B50)
-
- 5% Biodiesel and 47.5% GTL diesel addition to EN590 diesel
 - 10% Biodiesel and 45% GTL diesel addition to EN590 diesel
 - 30% Biodiesel and 35% GTL diesel addition to EN590 diesel

2.2. Fuel Composition

Identification and quantification of the diesels were analysed using Two dimensional gas chromatography (2-D GC) was used. The analyses were performed by the Analytical Science and Technology Group at the Sasol Technology, Research and Development Department in Sasolburg, South Africa. The analysis on every diesel was performed using a Pegasus 4D instrument equipped with time-of-flight mass spectrometric (TOF-MS) and FID detectors from Leco Corporation (St. Joseph, MI, USA). The first dimension column was a 30m x 250mm x 0.25mm RTX-Wax (Restek) with a temperature program of 40°C (0.2min), ramped at 2°C/min to 245°C. The second dimension column was a 1.8 m x 100mm x 0.1mm RTX-5 (Restek), and the second oven followed the first oven with a lead of 40°C. The modulation period was 7 seconds. A constant Helium carrier gas flow of 1.2mL/min was maintained with a split ratio of 400:1 and an injection volume of 0.1mL. TOF-MS was used for identification of compounds and the FID for quantification. TOF-MS and FID data were collected at 100 spectra (points)/sec.

2.3. Biodegradation

2.3.1. Theoretical Oxygen Demand & Biodegradation Calculations

Mineral oils, such as diesel fuels, can be assumed to have an empirical formula of C_nH_{n+2} ,

which is used to determine the theoretical oxygen demand (ThOD) for the complete biodegradation of diesel samples (Battersby, 2000). Two dimensional gas chromatography (2-D GC) was used to determine the hydrocarbon distribution of the various diesel samples. The average molecular weight and empirical formula was calculated from the chemical composition, which were, in turn, used to determine the ThOD according to the OECD 301F methodology (OECD 301F, 1992).

ThOD is defined as the total amount of oxygen required to oxidise a chemical completely, and is expressed as mg oxygen per mg chemical. The ThOD can be calculated using the elemental composition of the test chemical ($C_cH_hCl_{cl}N_nNa_{na}O_oP_pS_s$) by using the following formula (1).

$$\text{ThOD} = \frac{16 [2c + 1/2(h - cl - 3n) + 3s + 5/2p + 1/2na - o] \text{ mg/mg}}{\text{Molecular weight (MW)}} \quad (1)$$

The biodegradation in percentage of the ThOD was calculated using the following formulae (2 and 3).

$$\text{BOD} = \frac{(\text{mg O}_2 \text{ uptake in test flask} - \text{mean mg O}_2 \text{ uptake in blanks})}{\text{mg test substance in test flask}} \quad (2)$$

$$\% \text{ Biodegradation} = \frac{\text{BOD (mg O}_2\text{/mg test substance)}}{\text{ThOD (mg O}_2\text{/mg test substance)}} \quad (3)$$

2.3.2. Manometric Biodegradation Test

Biodegradability testing was carried out using a Coordinated Environmental Services (CES) aerobic respirometer according to the manometric respirometer (OECD 301F, 1992) method. A measured volume of inoculated mineral medium, containing a known concentration of the test substance, as the nominal sole source of organic carbon was used in the test. The return-water containing micro-organisms from a sewage treatment

facility that treats mainly domestic sewage (>90%) was used as the inoculum for the test. This was obtained from a Sasol One wastewater treatment works (WWTW) which is dedicated to treat domestic sewage.

The CES respirometer measured the oxygen consumption by the respiring micro-organisms in the test flasks and the instrument automatically logged the oxygen uptake every hour and replenished the oxygen used through electrolysis of copper sulphate. The carbon dioxide produced by the micro-organisms was absorbed from the headspace by a sodium hydroxide solution in a container inside flasks' the headspace. Degradation was monitored over a period of 28 days by determining the amount of oxygen consumed. The resolution of the respirometer was 0.02mg oxygen at standard temperature and pressure (STP).

The diesel samples were tested in duplicate at an average nominal concentration of 27mg/L (equivalent to 94 ± 4 mg/ThOD/L). The test flasks containing 1 L of mineral media were dosed with 27 ± 1 mg test samples absorbed onto 21mm Whatman GF/A glass fibre filters, weighed on a Sartorius balance with four decimals for accuracy. The test also contained duplicate blank flasks containing only mineral media, glass fibre filter paper and inoculum. In addition the tests also included a reference substance known to biodegrade rapidly, sodium acetate, in duplicate replicates which contained mineral media, glass fibre filter paper and 90 ± 1 mg of sodium acetate (equivalent to 70 ± 1 mg/ThOD/L) each.

The pass level for "ready biodegradability", when using respirometric test methods is to reach 60% of ThOD within 28 days. The pass values have to be reached within 10-days of the start of the degradation which is taken at the point when 10% of the substance has degraded. Chemicals that reach the pass level after 28 days are not deemed to be readily biodegradable. Although the test methodology rigorously indicates the pass level mentioned above for a substance to be classified "readily biodegradable", the classification in Annex VI to the European Dangerous Substances Directive (Council Directive 67/548/EEC, 2001), which specifies the classification and labelling of existing substances, also accepts that substances can be classified "ready biodegradable" when convincing scientific evidence is available to demonstrate that the substance can be degraded (biotically and/or abiotically) in the aquatic environment to a level of > 70% within a 28-day period.

For the test to be valid, the oxygen uptake of the inoculum blanks should not exceed 60mg/L in 28 days and the pH at the end of the test should be between 6 and 8.5. In addition, the reference substance must have reached 60% degradation by the 14th day of the test. All these criteria were met during these tests. No toxicity controls were performed.

2.4. Ecotoxicity Tests

A selection of aquatic sentinel organisms were used to assess the ecotoxicity of the various petroleum derived diesels, biodiesels and diesel blends. Aquatic ecotoxicity tests were selected because the current focus and need in the petroleum industry for classification and labelling is biological effect of standard ecotoxicity aquatic species. Ecotoxicological information on petroleum contamination of terrestrial habitats are widely available in research by Van Gestel *et al.* (2001), Namkoong *et al.* (2002), Salanitro *et al.* (1997), Dorn and Salanitro (2000) and Saterbak *et al.* (1999). Trends in regulatory policy and chemical risk assessment has been mainly based on aquatic toxicity although, in recent years, there has been more focus on terrestrial organisms and ecosystems, top predators, microorganisms in sewage treatment plants and the atmosphere (Van Leeuwen *et al.*, 1996).

The four acute aquatic ecotoxicity tests chosen for the present study were freshwater fish mortality using *Brachydanio rerio*, freshwater crustacean mobility using *Daphnia magna*, freshwater algal growth inhibition using *Pseudokirchneriella subcapitata*, and marine luminescent bacteria, *Vibrio fischeri*. All four ecotoxicity tests were conducted according to the standardised internationally accepted methodologies. The laboratory, although not ISO 17025 accredited, was participating in voluntary acute ecotoxicity Proficiency Testing Scheme (PTS) for *Poecilia reticulata*, *D. magna* and *V. fischeri* and the z-scores for those specific tests were within the ± 2 range.

2.4.1. Water Accommodated Fraction

The diesel products that were tested are poorly soluble in water and contained volatile substances, therefore it was not possible to prepare the required solutions of the product in water by direct addition of a measured quantity. Girling (1989) identified two types of test media that were used in previous work, water accommodated fractions (WAFs) and

dispersions. WAF is a media containing only the fraction of the substance which is retained in the aqueous phase after a period of mixing followed by a period of phase separation. Dispersions are media containing a quantity of the substance distributed homogeneously throughout the water by mixing or by the use of a chemical dispersion agent. The WAF was used to prepare the concentrations of the fuels used in the ecotoxicity tests in this study. WAFs can be prepared in two ways; one way by adding a single high loading rate and then diluting it to make a dilution series, or by preparing a WAF at each concentration of the range at different loading rates. CONCAWE (1993) recommended the latter method when using mixtures of samples with different water solubility, because the composition of the media will change both quantitatively and qualitatively as the loading rate changes.

Water accommodated fractions (WAFs) were prepared in sealed vessels with a small headspace according to the CONCAWE methodology for the ecotoxicological testing of petroleum products (CONCAWE, 1993). WAFs were prepared using 1 L reconstituted water (ISO 6341-1982) for the fish and crustacean tests. Reverse osmosis permeate (ROp) water from a Milli-Q system was used in the bacterial and algae tests.

The required amount of test substance was weighed on a Sartorius balance with four decimals accuracy onto microscope glass cover slips and added to 1 L reconstituted water or ROp. The vessels were sealed and vigorously shaken at 200 rpm for 24 hours on an orbital shaker. The mixtures were poured into separation funnels and left for two hours to separate. The water fractions were transferred to other vessels and used in the tests according to the applicable methods.

2.4.2. Fish, Acute Toxicity Test

The static non-renewal fish acute toxicity test was used to determine the toxicity of the fuels to freshwater *Brachydanio rerio* (Teleostei, Cyprinidae) (Hamilton-Buchanan), commonly known as the Zebrafish, according to the OECD 203 (1992) guidelines. The fish were obtained from Camcon CC, a fish wholesaler from Pretoria, SA that mainly supplies fish to pet shops. The exact age of the fish was not known, however, the fish used in the tests conformed to the guideline of total length of the fish of 2.0 ± 1.0 cm. The fish were exposed to nominal concentrations of the diesels in range-finding tests at loading rates of 100mg/L and 1 000mg/L.

Zebrafish were selected because they were easily procured, transported and acclimatised for the tests. All fish were in good health and acclimatised under laboratory conditions for 14 days or more before being used in the tests. Fish were kept in aerated fish tanks in temperature controlled water at 24 ± 1 °C and inside a temperature controlled room at 22 ± 1 °C. A photoperiod of 16 hours daily was maintained for both the test periods and acclimatisation periods. Fish were fed daily, except when a test was started, in which case the fish were not fed 24 hours prior to the start of the test. Mortalities were recorded at 24, 48, 72 and 96 hours. Reconstituted water according to the ISO 6341-1982 methodology, which is described in ANNEX 2 of the OECD 203 (1992) guidelines, was used as media for the preparation of the WAF as well as for the control. Seven fish per replicate were used to comply the the recommended maximum loading rate of 1 g fish/L. The temperature, pH and dissolved oxygen were measured at the beginning and end of the test. The fish were not fed during the tests. Tests were performed in triplicate with a minimum of seven fish per replicate and the controls.

For the tests to be valid, fish mortalities in the controls should not exceed 10% or one fish if less than ten are used. The test conditions were maintained for the duration of the test, dissolved oxygen should not be lower than 60% of the air saturation value for the duration of test and evidence that the concentration of the substance has been satisfactorily maintained for the duration of the test.

2.4.3. *Daphnia magna*, Acute Immobilisation Test

The static, non-renewal tests were conducted in accordance with the OECD 202 (2004) guidelines, the *Daphnia* sp., acute immobilisation test. The swimming capability or immobilisation of the crustacean, *D. magna* Straus, was used as the endpoint in determining the acute toxicity to the diesel products. *D. magna* neonates (<24 hours old) were exposed to the WAFs (100mg/L and 1 000mg/L) of the diesel products over a period of 48 hours. Exposures were performed in quadruplicate using five organisms in each replicate. Immobilised *D. magna* were identified if no movement was observed during a 15 second observation period and was recorded as such at 24 and 48 hours. The tests were deemed valid when less than 10% of the control daphnids were immobilised. The crustaceans were hatched from ephippia obtained from a standardised freshwater toxicity test kit, DAPHTOXKIT F™ Microbiotest, from ToxSolutions, Kits and Services cc in SA

that import the kits from a Belgian supplier, MicroBioTest Inc.

Reconstituted water according to the ISO 6341-1982 methodology, which is described in ANNEX 2 of the OECD 203 (1992) guidelines, was used as media for the preparation of the WAF as well as for the control. An amount of 2mL of media for each crustacean, a 16 hour photoperiod per day and constant water temperature of $22\pm 2^{\circ}\text{C}$ were maintained for the duration of the test. The *D. magna* neonates were not fed during the tests.

2.4.4. Algal Growth Inhibition Test

The algal growth inhibition test, OECD 201 (2006) guideline was used to assess the impact of the diesels to freshwater algae using *Pseudokirchneriella subcapitata* (formally known as *Selenastrum capricornutum*), ATCC 22662, CCAP 278/4, 61.81 SAG. In this static non-renewal test the growth of unicellular green algae is used to determine the effect of a test substance. This was conducted using the Algaltoxkit™ freshwater toxicity test with microalgae from the Laboratory for Biological Research in Aquatic Pollution (LABRAP) at the University of Ghent in Belgium.

For this test, WAFs with loading rates of 100mg/L and 1 000mg/L were prepared using ROp water. The algal beads from the Algaltoxkit™ were de-immobilised in the algal culturing media prescribed in the Algaltoxkit™ Standard Operating Procedure (2009). The Algaltoxkit™ technology is based on the measurement of the optical density of algal cell suspensions in disposable spectrophotometric cells of 10 cm path-length, also known as “long cells”. The measurement of the optical density was performed with a Jenway 6300 spectrophotometer (Jenway Ltd, England) with a 670nm filter.

To make up the required volume for the tests, 200mL of the respective WAF solutions were added into volumetric flasks. 1mL of the de-immobilised algal solution was added to the cells in the volumetric flasks and shaken vigorously to distribute the algae throughout the solution. The solutions were transferred to the long cells in 25mL aliquots of each WAF concentration in three replicates. The long cells were kept in a transparent holding tray in a random manner to prevent site-to-site differences during the incubation period. The holding trays were kept in a temperature controlled humidity chamber at $23\pm 2^{\circ}\text{C}$ and an illumination of 10 000 lux from white fluorescent lamps attached to the sides of the

chamber. The incubation period was 72 hours and the algal test solutions were resuspended once a day.

2.4.5. BioTox™ *Vibrio fischeri* Bioluminescence Inhibition Test

The BioTox™ Kit was used to determine the toxicity of water soluble samples according to the ISO 11348-3:1999 method (ISO 11348-3, 1999). As the diesel products were poorly soluble, WAFs were used to assess the effect on the luminescent bacteria. The toxicity potential of the respective diesels was determined by the reduction in light emission of the luminescent bacteria, *Vibrio fischeri*, and was measured with a BioOrbit™ luminometer. The bacteria was obtained from a standardised marine toxicity test kit, BioTox™ luminometric testing system, from ToxSolutions, Kits and Services cc in South Africa that import the kits from a Belgian supplier.

The WAFs that were prepared with ROp water and loading rates of 100mg/L and 1 000mg/L diesel were used for the tests. The salinity of the samples was adjusted by adding a 2% w/v sodium chloride solution to simulate seawater salinity. The freeze-dried *Vibrio fischeri* was reconstituted with the reagent diluent provided in the BioTox™ Kit at 4°C for a period of 30 minutes and then stabilised at 15°C for an additional 30 minutes.

The reconstituted *V. fischeri* was transferred in 500µL aliquots in cuvettes kept at a constant temperature of 15°C in a dry block incubator. The luminescence of the *V. fischeri* containing cuvettes was measured using a BioOrbit™ luminometer followed by the addition of 500µL saline adjusted diesel WAF samples. The samples were performed and measured in duplicate with duplicate control samples containing saline adjusted ROp water instead of WAF samples. The cuvettes were incubated for 30 minutes with luminescence measurements taken at 15 and 30 minute intervals respectively.

2.5. Quantitative structure-activity relationship

2.5.1. Ecotoxicity estimation

The latest EPI suite QSAR version and the version that was used in all the estimations was downloaded and installed on Thursday, August 06, 2009, 9:29:13 AM. The methods used for obtaining the hazard properties of the fuels are described in Fisk (2005). The molecular structures, molecular weight, water solubility and octanol/water partition

coefficient were estimated by using the models in EPI Suite™ V4.0 according to the 13 different models. The percentage contribution of each constituent was included from the 2D-GC analysis and the hydrocarbon/water partition coefficient values were calculated from the octanol/water partition coefficient. The data was organised according to carbon number, molecular formula, CAS number and the mass percentage contribution to the fuel. The molecular weights for the identifiable individual constituents were obtained from the EPI Suite™ V4.0 databank or calculated from molecular structure formulae.

The Fisk Ecotoxicity Estimation Model (FEEM), according to the principles described in Fisk (2005) was used to determine the species-specific acute toxicity units (TUa) values. This model elegantly integrates the methodologies of the ECOSAR model from EPI Suite™ V4.0 with the KOWWIN to estimate organic concentrations of the respective individual constituents of the diesel WAFs. The L(E)C₅₀ concentrations are then estimated using the generic equation (EQ. 4) from ECOSAR to predict acute toxicity.

$$\log(\text{LC}_{50}, \text{EC}_{50} \text{ or } \text{NOEC}) = a \log(\text{Kow}) + b \quad (4)$$

where a and b are constants equal to the slope and the intercept on the y-axis of the regression equation derived from the ECOSAR QSAR for predicting the acute toxicity of non-polar narcotics.

The reciprocal of the L(E)C₅₀ values are calculated to convert it to toxicity units. The TUa of the individual hydrocarbon constituents can be summed to give a total TUa associated with the specific species and diesel. If the TUa of that specific species exceed 1 unit, an adverse effect is expected. Because the generally accepted mechanism for toxicity of organisms to hydrocarbons is non-specific narcosis, the toxicity of individual compounds in the mixture is believed to be additive, the individual TUa values were summed. The effect of the major hydrocarbon group contribution to toxicity can be evaluated by using the following groups: paraffins, branched paraffins, monocyclic paraffins, bicyclis paraffins, polycyclic praffins, mononuclear aromatics, bicyclis aromatics and polycyclic aromatics.

2.5.2. Biodegradation estimation

The biodegradation was estimated via the BIOWIN V.4.10 is the EPI Suite prediction software program. It contains six different methods, Biowin 1 – 6, each calculating the

probability of biodegradation (Table 5). The individual constituents of the fuels were classified according to the Biowin 3 output values from the Ultimate Biodegradation Models where substances with values ≥ 3.3 were ascribed Readily Biodegradable, meeting the 10-day window (RB); substances with values ≥ 3.0 were ascribed Readily Biodegradable, not meeting the 10-day window (RBN); substances with values ≥ 2.8 were ascribed Inherently Readily biodegradable (IB), fulfilling the criteria; and substances with values < 2.8 were ascribed Inherently Readily biodegradable, Not fulfilling the criteria (IBN). These cut-off values were adopted from Girling and Fisk (2006) who derived it from Solano-Serena et al. (2000) and Hansveit (1992) experimental data for hydrocarbon substances that were estimated using the Biowin 3 Ultimate model.

Table 5: BIOWIN V.4.10 software programs for the estimation biodegradability probability. The Biowin 7 (Anaerobic Biodegradation Model) was not included in this study.

Method	Name	Output	Output interpretation
Biowin 1 and 2	Linear and Non-linear Biodegradation Models	Numerical and Unitless Indicates probability of degradation without specifying a timeframe	Probability ≥ 0.5 indicates Biodegradability Fast Probability < 0.5 indicates Does NOT Biodegrade Fast
Biowin 3 and 4	Primary and Ultimate Biodegradation Models	Numerical and Unitless Indicates a point on a scale of timeframes for degradation	Result Classification (Primary and Ultimate): 5.00 = hours; 4.00 = Days; 3.00 = Weeks; 2.00 = Months; 1.00 = Longer
Biowin 5 and 6	Linear and Non-linear MITI* Biodegradation Models	Numerical and Unitless Indicates probability of degradation without specifying a timeframe	Probability ≥ 0.5 indicates Biodegradability Fast Probability < 0.5 indicates Does NOT Biodegrade Fast

* Ministry of International Trade and Industry used to be an agency that funded research in the Japanese government. Its role was taken over by the Ministry of Economy, Trade and industry (METI).

The estimation models were only applied to the complete diesels and diesel blending components. The experimental blends of biodiesel, GTL diesel and EN590 were calculated based on the ratio of the diesels.

- 3 -

Results

3.1. Fuels

Visually the WAFs of the complete diesels that were tested did not differ and it resembled clear water, with the exception of the biodiesel. The biodiesel WAF was visually distinctly different to the other diesels by its milky white appearance. The diesel blends containing biodiesel had varying levels of a milky or cloudy appearance.

3.2. Fuel Chemical Composition

With the exception of biodiesel, the results from the 2-D GC analysis on the experimental diesels indicated that the complete diesels contained predominantly hydrocarbons (HCs) in the $C_8 - C_{26}$ range.

Results from the 2-D GC analysis on the experimental diesels are presented in Figures 2 – 9. A summary of the results is presented in Table 6.

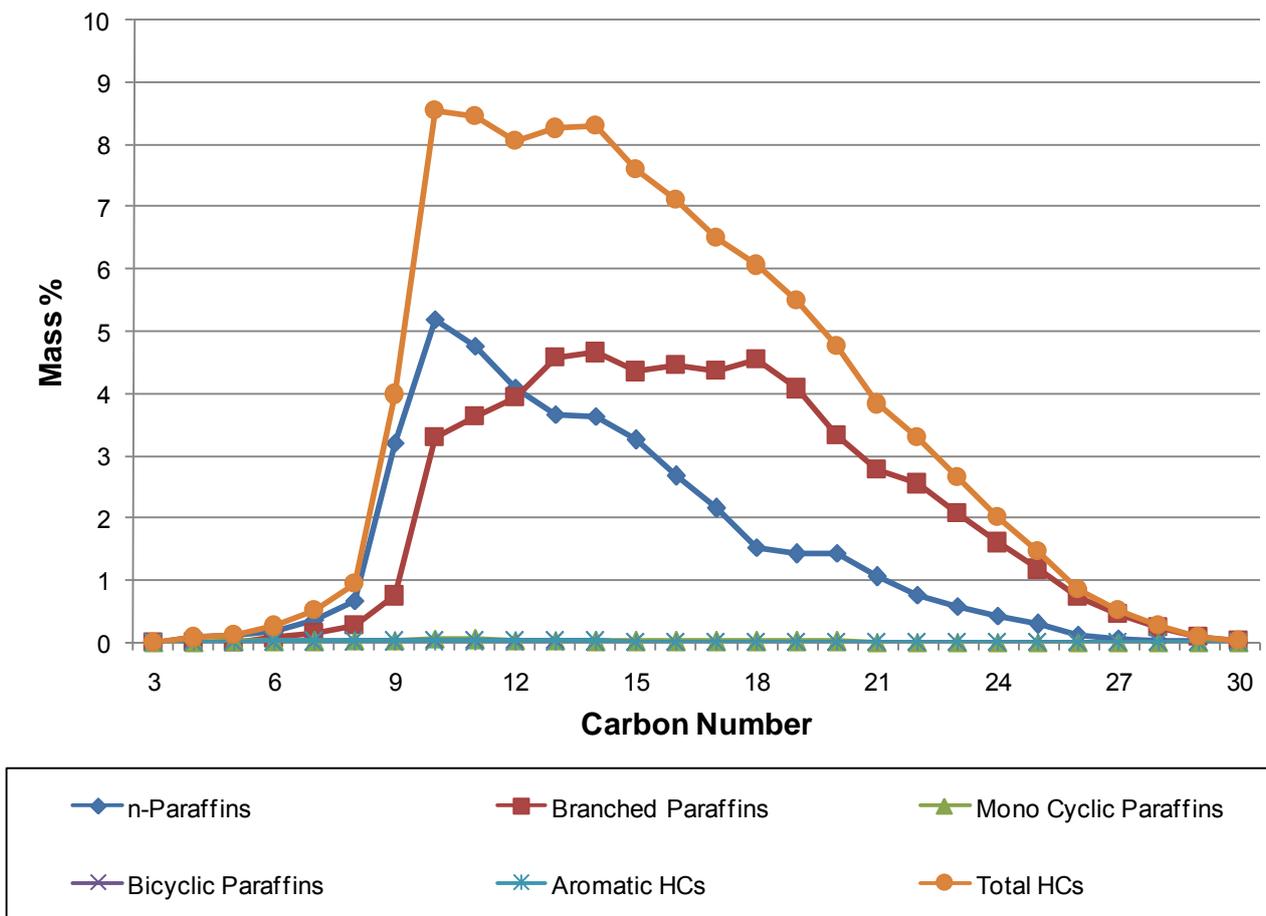


Figure 2: Graphic illustration of 100% GTL diesel hydrocarbon constituent distribution from the 2-D GC analysis.

The GTL diesel consisted almost entirely of normal and primary branched alkanes, with less than 0.5% cyclic alkanes and aromatic HCs combined. Overall, the hydrocarbon distribution peaked between C_{10} and C_{14} . The n-paraffins peaked at C_{10} and the branched paraffins peaked between C_{13} and C_{18} . Cyclic paraffins and aromatic hydrocarbons were almost completely absent in GTL diesel.

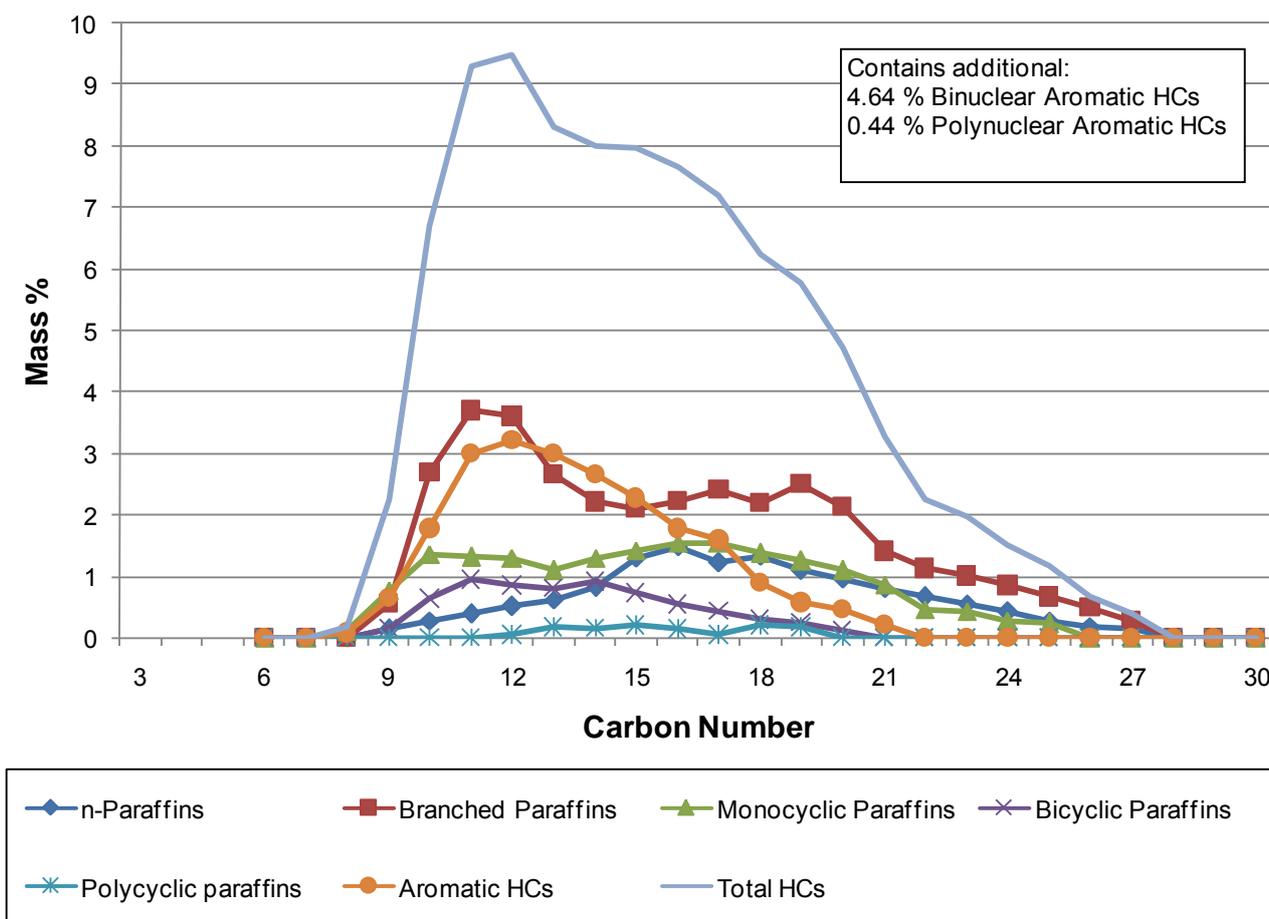


Figure 3: Diagrammatic illustration of the hydrocarbon constituent distribution of Natref diesel blend earmarked for vehicle fuel from the 2-D GC analysis.

The Natref diesel consisted of a mixture of HCs of which normal paraffins, branched paraffins, monocyclic paraffins and mono-aromatic HCs represented the main chemical groups of the diesel constituents. Aromatic HCs represented approximately 27% of the diesel of which mono-aromatic HCs were the most prevailing at approximately 22% of the total mass percentage of the diesel. Approximately 5% bi- and polynuclear aromatics are present in the diesel. Overall, the hydrocarbon distribution peaked at C₁₂.

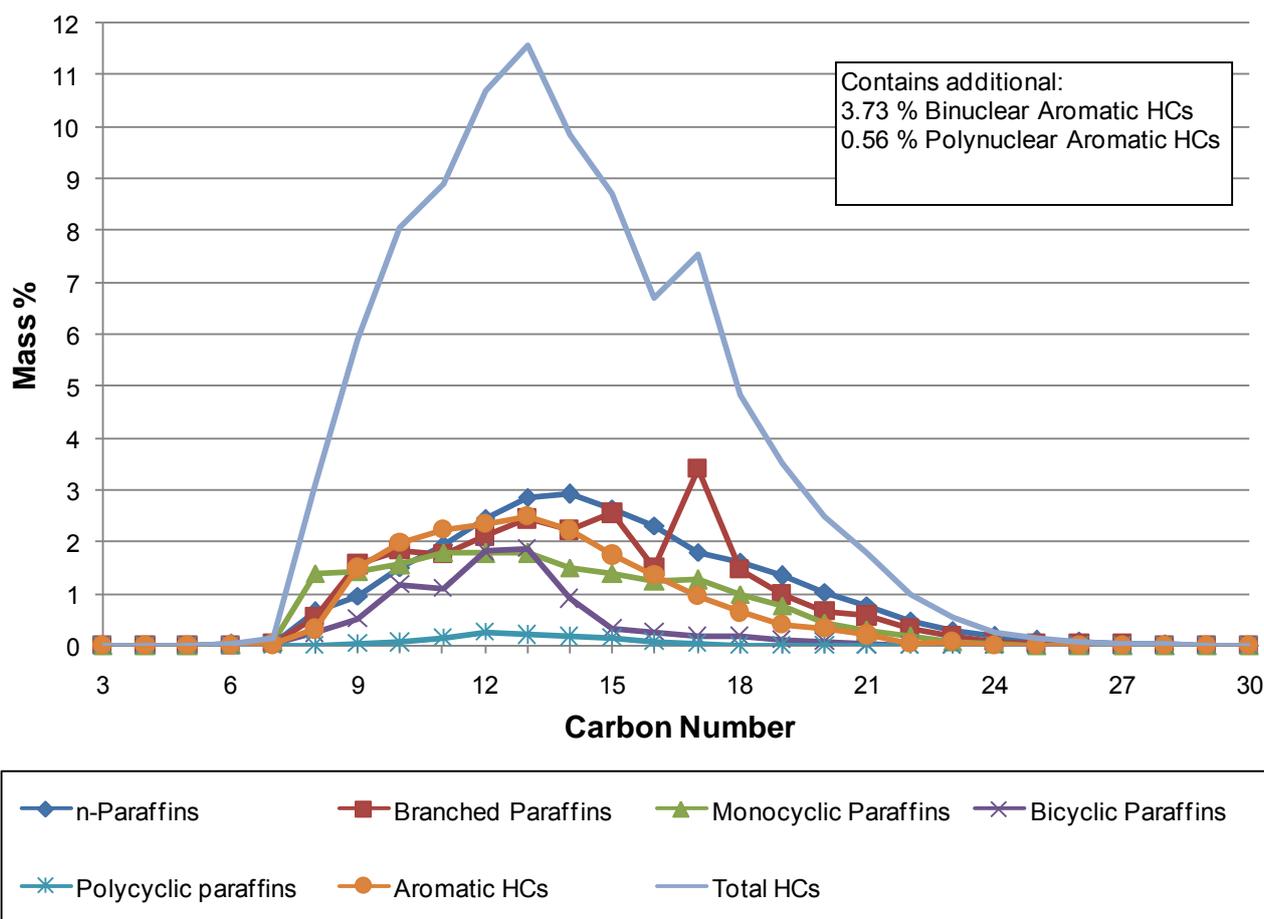


Figure 4: Diagrammatic illustration of the hydrocarbon constituent distribution of European specification EN590 diesel blend earmarked for European use from the 2-D GC analysis.

The European reference diesel, EN590, contained mainly normal paraffins, branched paraffins, monocyclic paraffins and mono-aromatic HCs. The total aromatic HCs contribution was approximately 23% of the total mass percentage of the diesel. Overall, the hydrocarbon distribution peaked at C₁₃. Approximately 4% of the total hydrocarbons were aromatic constituents.

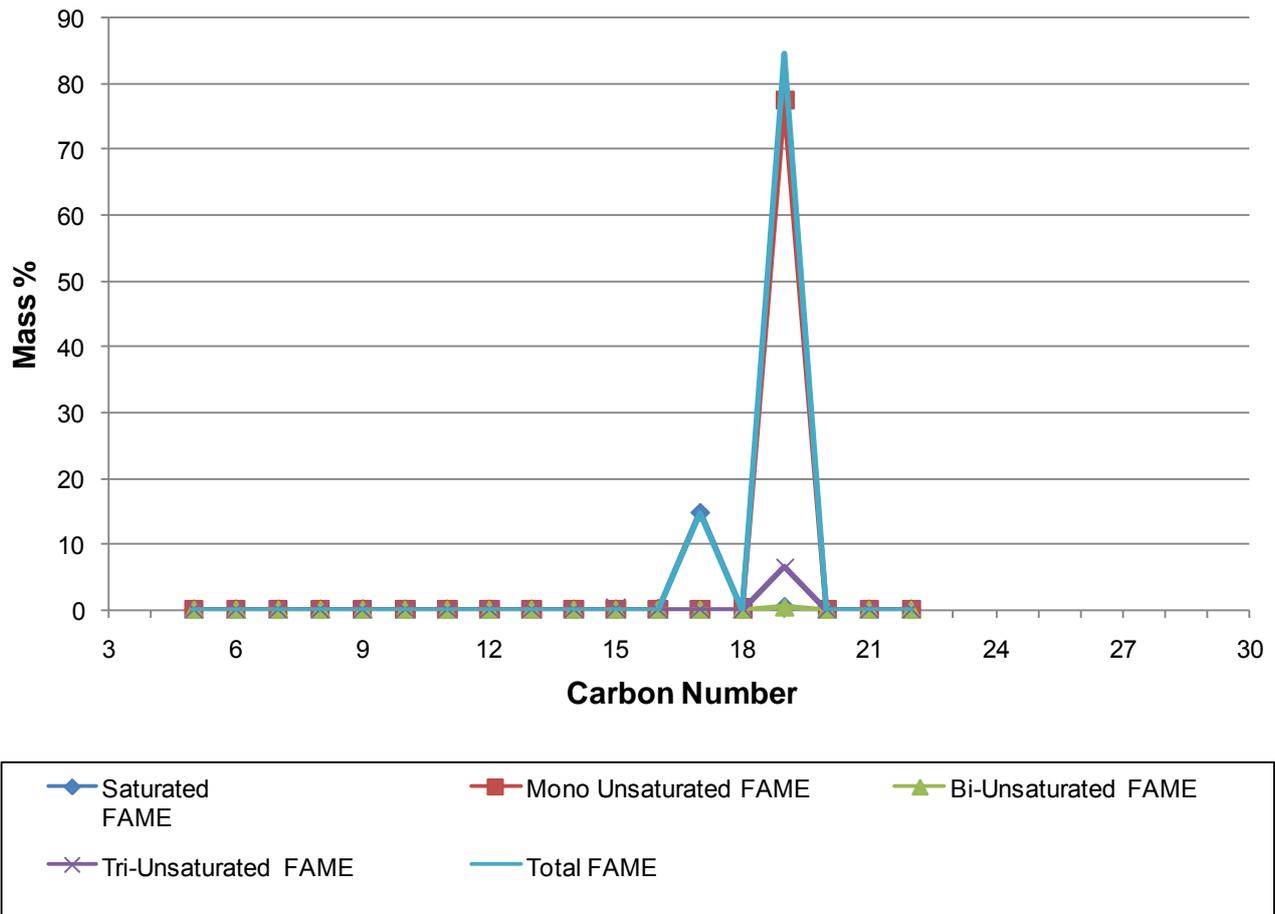


Figure 5: Diagrammatic illustration of the hydrocarbon constituent distribution of soybean derived biodiesel from the 2-D GC analysis.

The biodiesel contained saturated and unsaturated fatty acid methyl esters (FAME), with the major constituents being C₁₇ saturated FAME and C₁₉ mono-unsaturated FAME, with the former making up approximately 15% and the latter approximately 78% of the biodiesel.

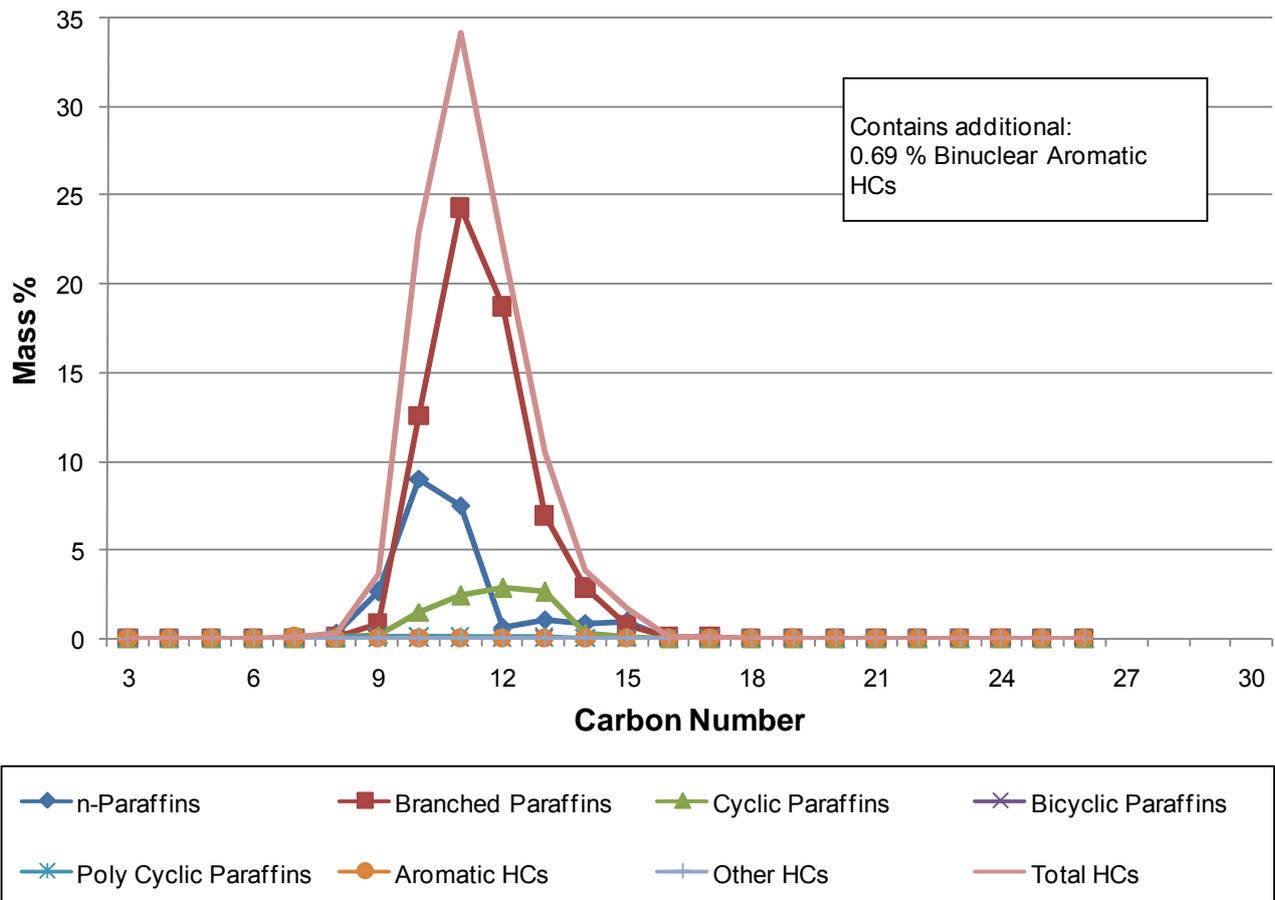


Figure 6: Diagrammatic illustration of the hydrocarbon constituent distribution of DHT Light diesel blend produced in Secunda earmarked for South African use from the 2-D GC analysis.

DHT Light Diesel Blending Material (DBM) had a narrow HCs range between C_8 and C_{15} . Three main chemical groups were represented which included normal-, branched- and monocyclic paraffins. Branched paraffins made up more than 66% of the total mass percentage of the diesel. Overall, the hydrocarbon distribution peaked at C_{11} . This DBM contained less than 1% aromatic HCs.

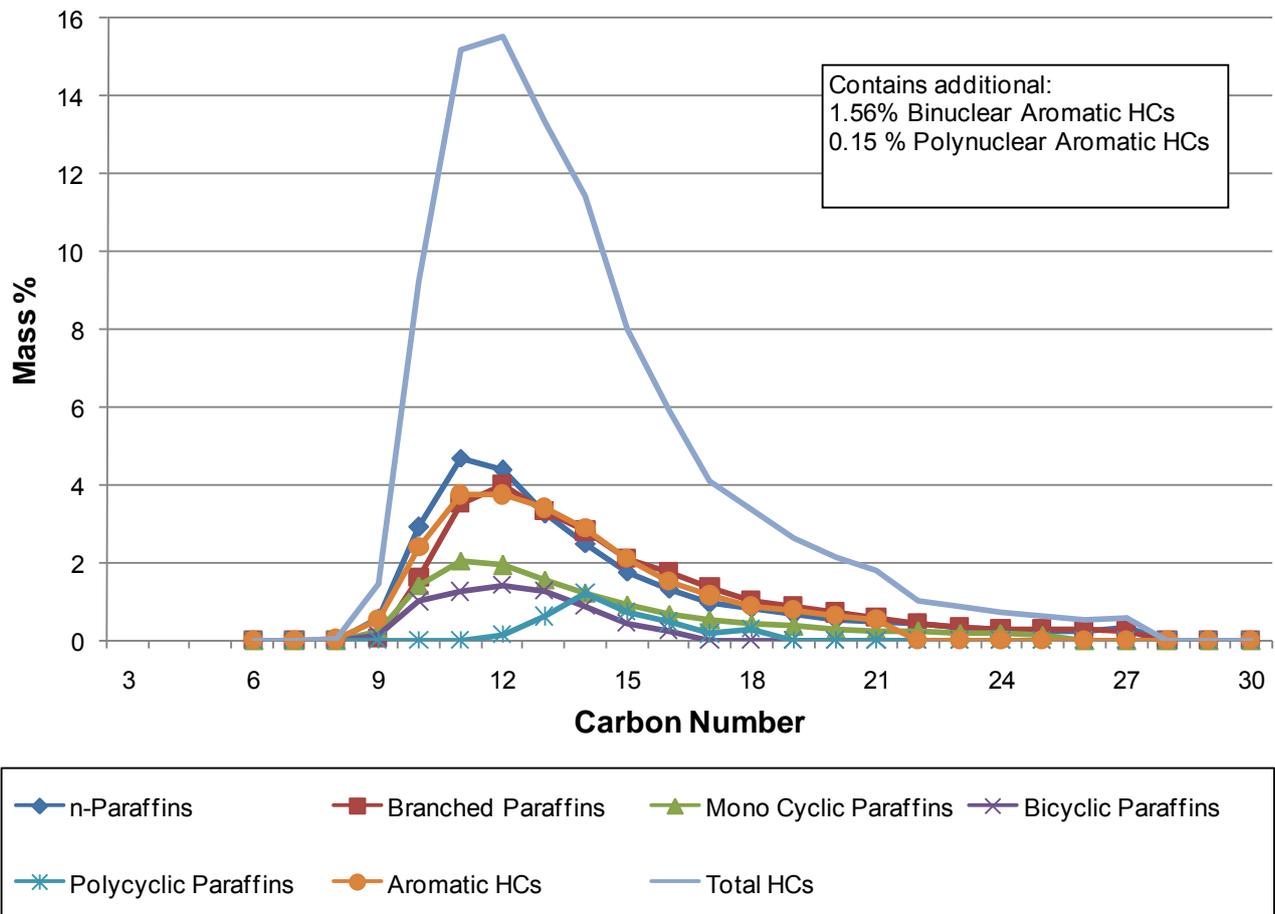


Figure 7: Diagrammatic illustration of the hydrocarbon constituent distribution of DSC Heavy diesel blend produced in Secunda earmarked for South African use from the 2-D GC analysis.

DSC Heavy DBM consisted mainly of normal -, branched-, monocyclic paraffins and aromatic HCs. Aromatic HCs represented approximately 26% of the diesel of which mono-aromatic HCs made up approximately 24% of the total mass percentage of the diesel. Overall, the hydrocarbon distribution peaked at C₁₁/C₁₂.

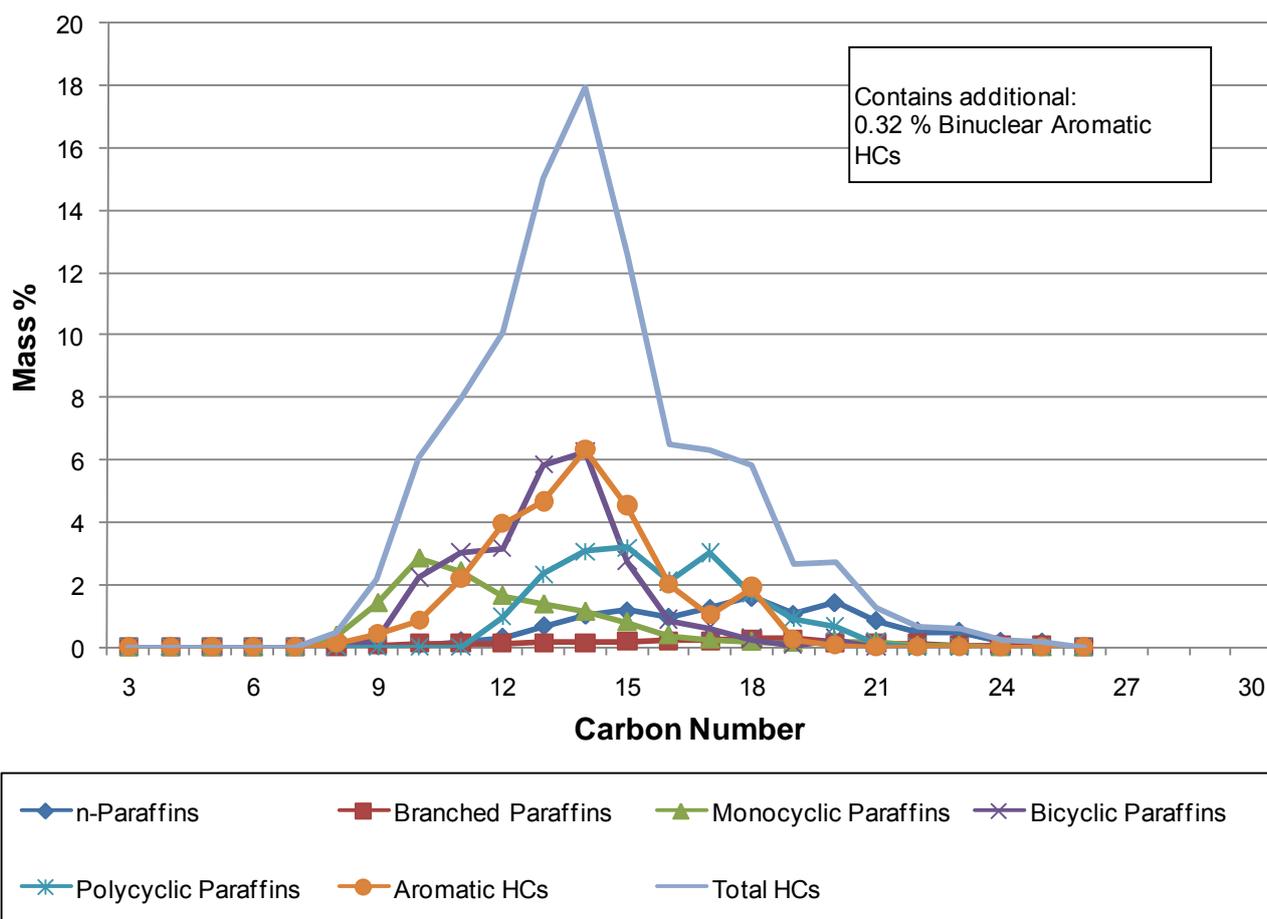


Figure 8: Diagrammatic illustration of the hydrocarbon constituent distribution of Creosote diesel produced in Secunda and used as diesel blending material from the 2-D GC analysis.

Creosote DBM consisted mostly of cyclic paraffins and constituted approximately 57% of the total mass percentage of the DBM. Aromatic HCs also made up a large percentage of the total mass percentage of the DBM at approximately 29%. Normal paraffins made up a small percentage of the total mass percentage of the DBM at approximately 12%. Overall, the hydrocarbon distribution peaked at C₁₄.

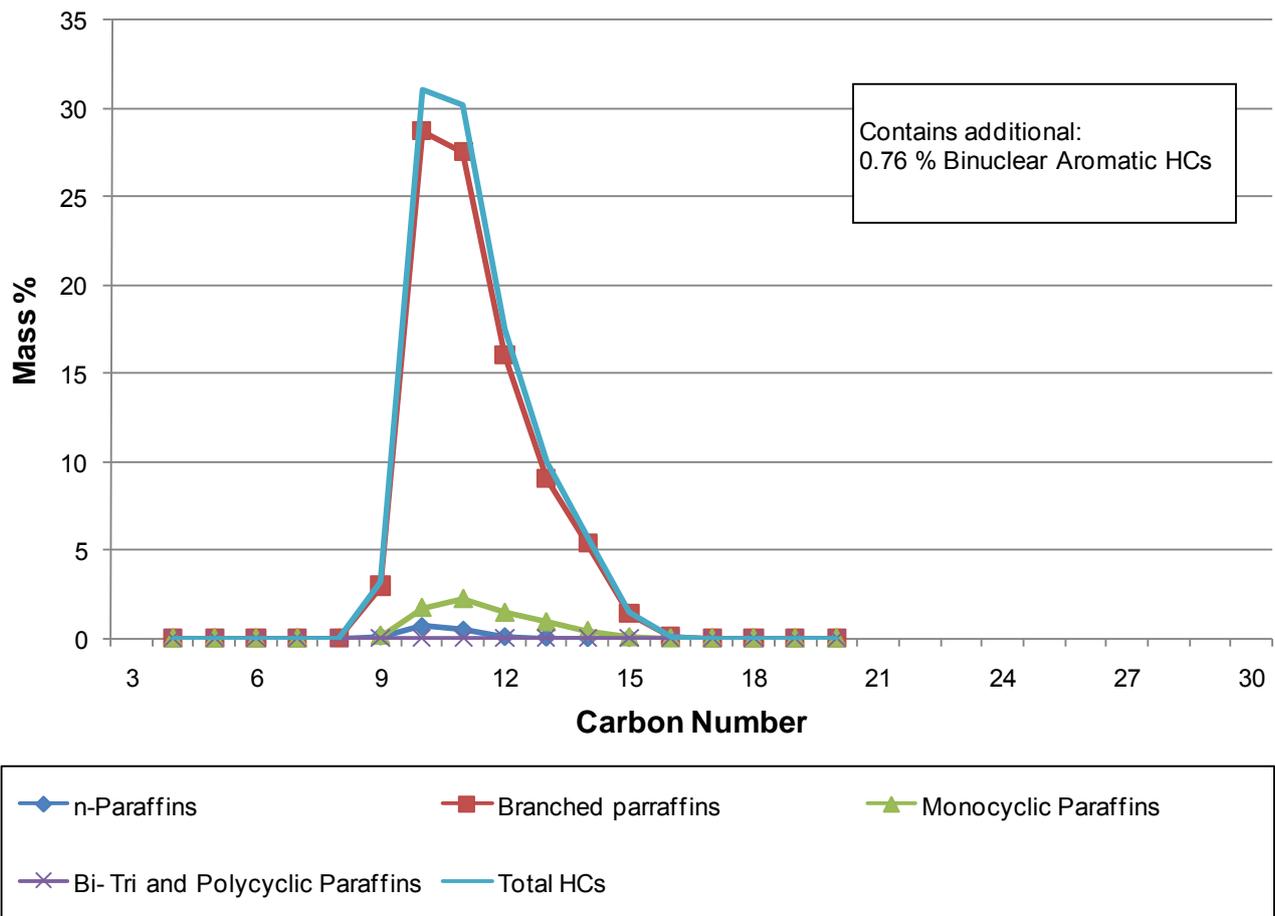


Figure 9: Diagrammatic illustration of the hydrocarbon constituent distribution of CatPoly diesel produced in Secunda and used as diesel blending material from the 2-D GC analysis.

CatPoly DBM consisted mainly of branched paraffins that constituted more than 91% of the total mass percentage of the DBM in a carbon range of $C_{10} - C_{14}$. No aromatic HCs were detected in this DBM. Overall, the hydrocarbon distribution peaked at C_{10}/C_{11} .

Table 6: Chemical composition of the complete diesels that were analysed using 2-D GC analysis. Chemical groups were used to simplify analysis of the data. The contribution of each chemical group is in mass percentage.

Chemical Group	GLT Diesel	Natref Diesel	EN590 Ref. Diesel	Soya Biodiesel	DHT Light DBM	DSC Heavy DBM	Creosote DBM	CatPoly DBM
n-Paraffins	41.59	13.01	25.68	-	22.65	26.41	11.80	1.30
Branched Paraffins	58.08	34.64	24.08	-	66.80	25.27	2.34	91.03
Monocyclic Paraffins	0.22	17.60	17.74	-	9.69	12.45	13.52	6.91
Bicyclic Paraffins	0.08	6.55	8.57	-	0.07	6.53	25.62	0.76
Polycyclic Paraffins	-	1.06	0.99	-	0.09	3.54	18.24	-
Mono-Aromatic	0.03	22.06	18.65	-	0.01	24.09	28.24	-
Bi-Aromatics	-	4.64	3.73	-	0.69	1.56	0.32	-
PolyAromatics	-	0.44	0.56	-	-	0.15	-	-
Saturated Esters	-	-	-	15.35	-	-	-	-
Mono Unsaturated Esters	-	-	-	77.59	-	-	-	-
Bi-Unsaturated Esters	-	-	-	0.47	-	-	-	-
Tri-Unsaturated Esters	-	-	-	6.59	-	-	-	-
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

DBM = Blending Material

3.3. Biodegradation

3.3.1. Theoretical Oxygen Demand & Biodegradation Calculations

The theoretical oxygen demand (ThOD) for the diesels and diesel blends were calculated from their respective hydrocarbon molecule substitutes presented in Table 7.

Although mineral oils, such as diesel fuels, can be assumed to have an empirical formula of C_nH_{n+2} , which is used to determine the theoretical oxygen demand (ThOD) for the complete biodegradation of diesel samples (Battersby, 2000), this research aimed at calculating the ThOD more accurately by using the hydrocarbon constituents of the various diesel samples. The average molecular weight and empirical formula was calculated from the chemical composition, which were, in turn, used to determine the ThOD according to the OECD 301F methodology (OECD 301F, 1992).

The ThOD of the individual hydrocarbon molecules could not be used because diesel is a complex mixture of millions of molecules and hence hydrocarbon substitute molecules were used for particular groups of hydrocarbons found in the diesels. The results are presented in Tables 7 and 8.

Table 7: The hydrocarbon molecule substitutes for the individual constituents that constituted the diesels and diesel blends that were used to calculate the more ThOD accurately.

Generic Name	Substitute molecule	ThOD
n-Paraffin	C16 Alkane (n-Hexadecane)	3.46
Branched Paraffins	C11 branched Alkane (2-methyldecane)	3.48
Monocyclic Paraffins	C10 monocyclo-alkane (Cyclodecane)	3.42
Bicyclic Paraffins	C10 bicyclo-alkane (Decahydronaphthalene)	3.36
Polycyclic Paraffins	C14 tricyclo-alkane	3.33
Mononuclear Aromatic	C6 aromatic (Benzene)	3.07
Binuclear Aromatic	C10 bi-aromatic (Naphthalene)	3.00
Polynuclear Aromatic	C14 poly-aromatic (Phenanthrene)	2.96
Saturated methyl ester	C17 FAME (Methyl hexadecanoate)	2.70
Mono-unsaturated methyl ester	C19 FAME (Methyl oleate)	2.91
Bi-unsaturated methyl ester	C19 FAME (Methyl linoleate)	2.88
Poly-unsaturated methyl ester	C19 FAME (Methyl linnolenate)	2.84
Sodium Acetate	N/A	0.78

Table 8: The calculated theoretical oxygen demand (ThOD) of the diesel fuels that were tested for biodegradation.

Diesel	Theoretical Oxygen Demand [mg O ₂ /mg substance]
Sasol GTL 100% diesel	3.47
Sasol Natref Final Diesel 100%	3.35
EN590 diesel	3.36
Biodiesel100 (Soybean derived fatty acid methyl esters (FAME))	2.87
DHT Light Secunda diesel	3.47
DSC Heavy Secunda diesel	3.30
Creosote diesel blending component	3.28
CatPoly diesel blending component	3.45
5% Biodiesel addition to EN590 diesel	3.33
10% Biodiesel addition to EN590 diesel	3.31
30% Biodiesel addition to EN590 diesel	3.21
0.1%Biodiesel addition to GTL diesel	3.47
0.2% Biodiesel addition to GTL diesel	3.47
0.4% Biodiesel addition to GTL diesel	3.47
0.8% Biodiesel addition to GTL diesel	3.47
1.6% Biodiesel addition to GTL diesel	3.46
3.2% Biodiesel addition to GTL diesel	3.45
6.4% Biodiesel addition to GTL diesel	3.43
12.5% Biodiesel addition to GTL diesel	3.40
25% Biodiesel addition to GTL diesel	3.32
50% Biodiesel addition to GTL diesel	3.17
5% Biodiesel and 47.5% GTL diesel addition to EN590 diesel	3.39
10% Biodiesel and 45% GTL diesel addition to EN590 diesel	3.36
30% Biodiesel and 35% GTL diesel addition to EN590 diesel	3.25

3.3.2. Manometric Biodegradation Test

Biodegradability testing was carried out using a CES aerobic respirometer according to the manometric respirometer (OECD 301F, 1992) method using a CES respirometer to measure the oxygen consumption by the respiring micro-organisms in the test flasks.

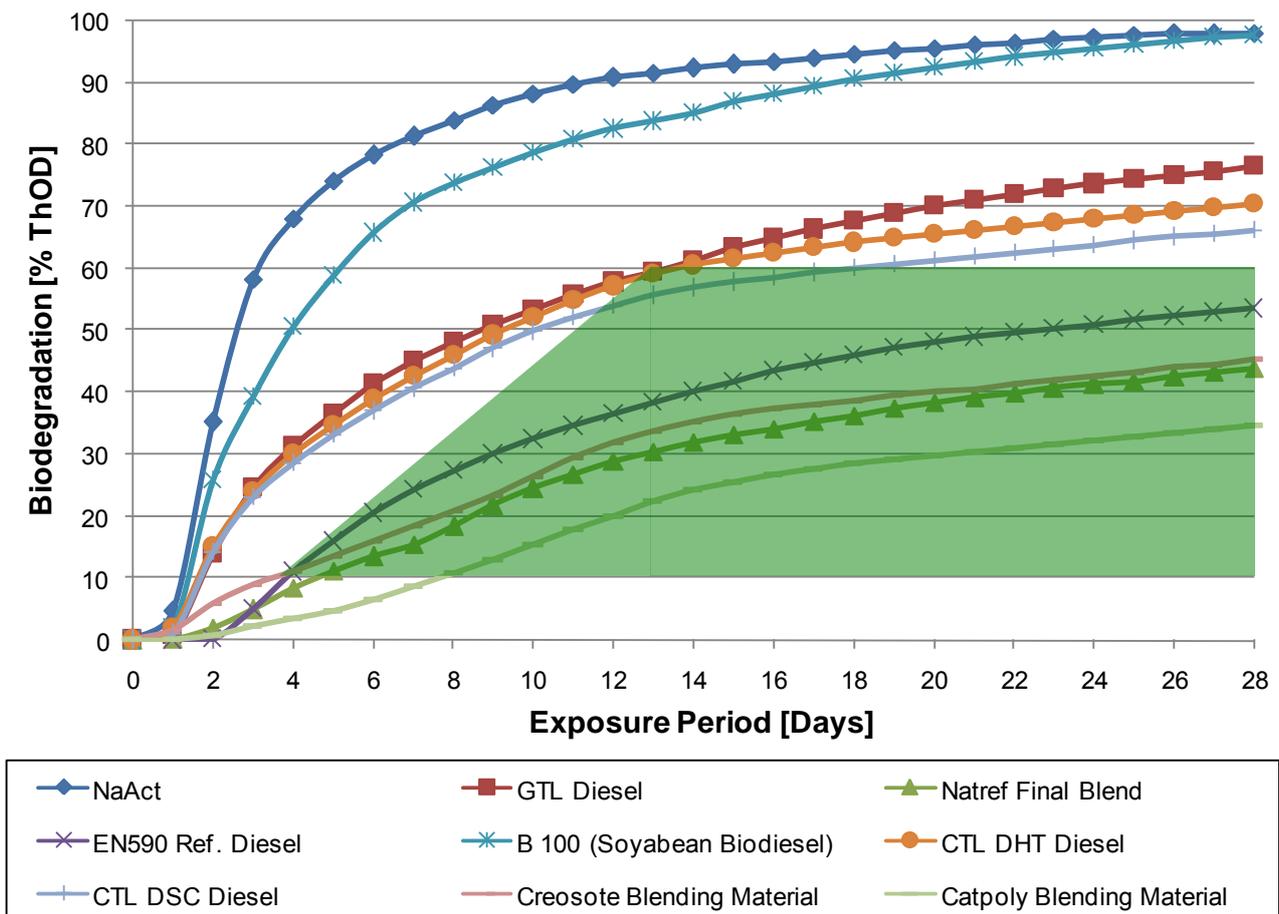


Figure 10: Biodegradability of complete diesels and diesel blending material measured by the respirometric “ready biodegradability” test. The shaded area represents the limit for the 10-day window.

In Figure 10 it is apparent that the neat biodiesel (B 100) diesel and GTL diesel biodegraded more than the other diesels and diesel blending material following 97.8% and 76.7% degradation respectively after 28 days. Only the biodiesel passed the criteria for “readily biodegradability” as prescribed by the test procedure by achieving 60% biodegradation (based on ThOD) within 10 days of reaching 10% degradation (also known as the 10-day window period) (OECD 301F, 1992). Although the GTL did not satisfy the 10-day window criteria set out in the method, it did achieve greater than 70% degradation

(based on% ThOD) within the 28 days of the test, which, according to the classification in Annex VI to the Dangerous Substances Directive (Council Directive 67/548/EEC, 2001), can be accepted as being “readily biodegradable (Table 9). The crude derived diesels and synthetic diesel blending material could not meet the criteria for readily biodegradability and could be regarded as “inherently biodegradable”.

Table 9: Biodegradation of complete diesels and diesel blending material at 12 and 28 days.

Complete Diesel or Blending Material	Biodegradation [% ThOD ± StDev]	
	12 Days	28 Days
Sasol GTL 100% diesel	57.86 ± 0.90	76.7 ± 0.90
Sasol Natref Final Diesel 100%	28.86 ± 2.65	43.92 ± 1.56
EN590 diesel	36.52 ± 0.61	53.57 ± 1.61
Biodiesel100 (Soybean derived)	82.64 ± 2.74	97.85 ± 1.98
DHT Light Secunda diesel	57.26 ± 3.83	70.59 ± 3.32
DSC Heavy Secunda diesel	54.11 ± 7.83	66.32 ± 5.01
Creosote diesel blending component	31.91 ± 3.28	45.37 ± 1.76
CatPoly diesel blending component	20.06 ± 0.76	34.74 ± 2.07

Biodiesel/EN590 diesel mixtures were prepared to illustrate the effect of biodiesel addition to conventional diesel and is illustrated in Figure 11.

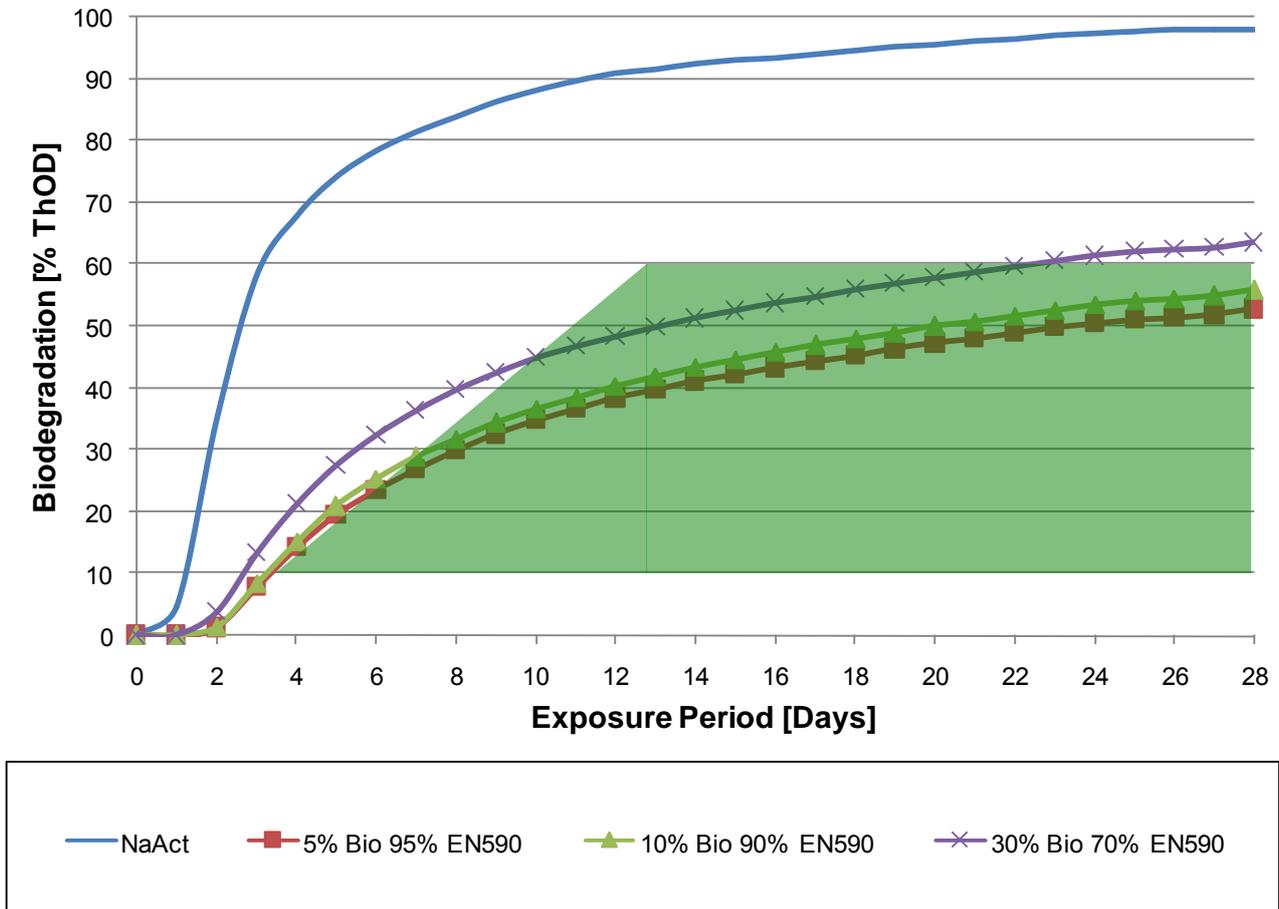


Figure 11: Biodegradation of biodiesel and commercial diesel (EN590 European diesel) blends by the respirometric “ready biodegradability” test. The shaded area represents the limit for the 10-day window.

None of the diesel blends passed the 10-day window criteria or that of the classification in Annex VI to the Dangerous Substances Directive (Council Directive 67/548/EEC, 2001). Since significant degradation did occur, these diesel blends can be considered “inherently biodegradable”. It was observed that with increasing biodiesel addition, biodegradability increased (Table 11).

Table 10: Biodegradation of Biodiesel / EN590 blends at 12 and 28 days.

Biodiesel / EN590 diesel blends	Biodegradation [% ThOD ± StDev]	
	12 Days	28 Days
5% Biodiesel addition to EN590 diesel	38.43 ± 1.3	52.82 ± 1.9
10% Biodiesel addition to EN590 diesel	40.37 ± 2.3	55.88 ± 3.6
30% Biodiesel addition to EN590 diesel	48.43 ± 1.8	63.53 ± 3.2

The biodegradability of GTL diesel and biodiesel blends are illustrated in Figure 12 and Table 11.

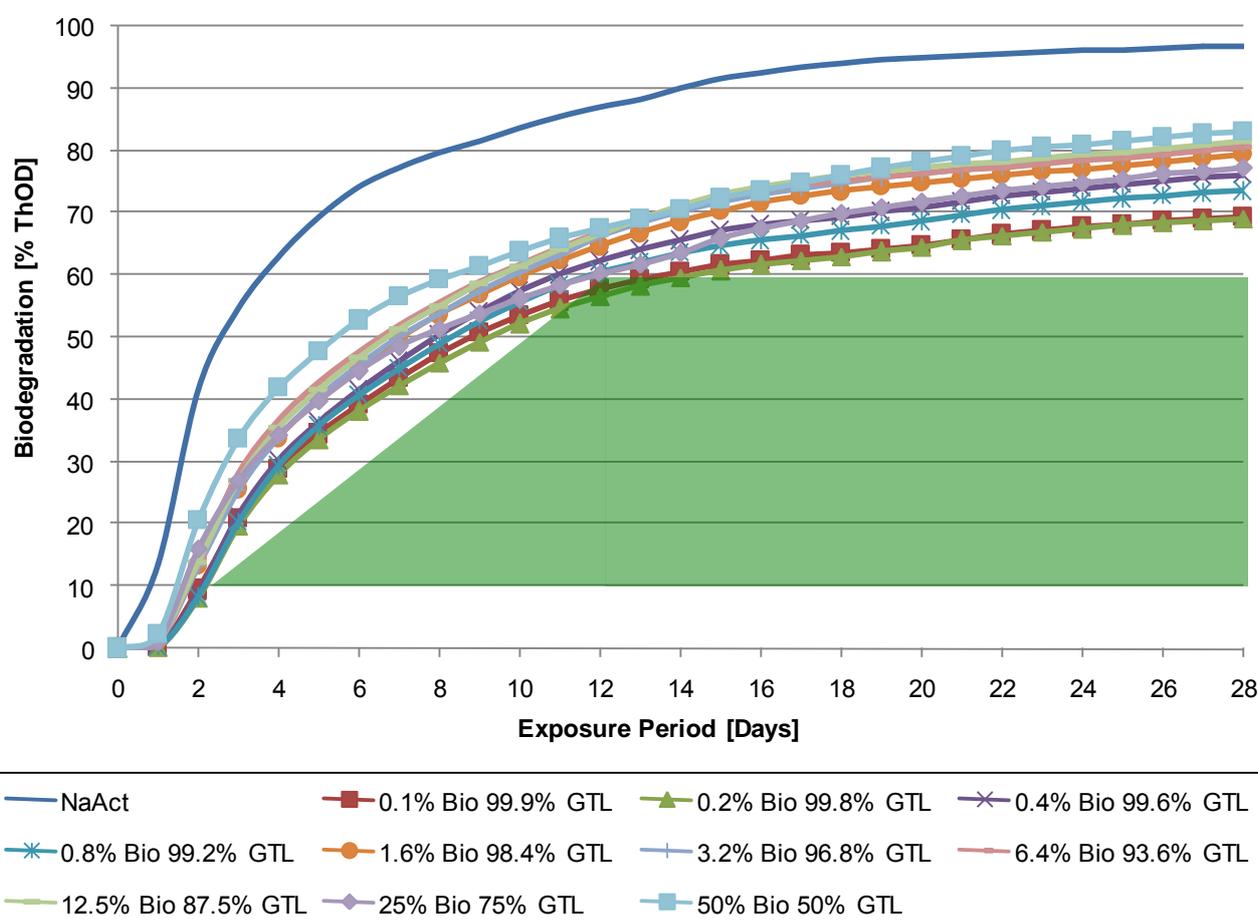


Figure 12: Biodegradation of biodiesel and GTL diesel blends by the respirometric “ready biodegradability” test. The shaded area represents the limit for the 10-day window.

Biodegradability of the biodiesel/GTL diesel blends showed high biodegradability rates when compared to the biodiesel/EN590 blends. All the diesels achieved greater than 60% biodegradation after 12 days, except the first two blends that contained 0.1% and 0.2% biodiesel respectively.

Table 11: Biodegradation of Biodiesel / GTL Diesel blends at 12 and 28 days.

Biodiesel / GTL diesel blends	Biodegradation [% ThOD ± StDev]	
	12 Days	28 Days
0.1% Biodiesel addition to GTL diesel	58.39 ± 1.4	69.43 ± 4.2
0.2% Biodiesel addition to GTL diesel	57.61 ± 4.8	69.08 ± 4.1
0.4% Biodiesel addition to GTL diesel	62.35 ± 6.3	76.20 ± 3.9
0.8% Biodiesel addition to GTL diesel	60.60 ± 2.7	73.55 ± 7.7
1.6% Biodiesel addition to GTL diesel	64.68 ± 3.6	79.48 ± 2.1
3.2% Biodiesel addition to GTL diesel	66.20 ± 5.4	80.90 ± 3.6
6.4% Biodiesel addition to GTL diesel	66.77 ± 3.0	80.64 ± 4.1
12.5% Biodiesel addition to GTL diesel	68.00 ± 2.5	81.46 ± 4.0
25% Biodiesel addition to GTL diesel	69.16 ± 2.1	77.42 ± 2.5
50% Biodiesel addition to GTL diesel	74.18 ± 4.4	83.21 ± 3.2

The effect of biodiesel addition on the biodegradation rate of the GTL-derived diesel blends is illustrated in Figure 13 by regression analysis.

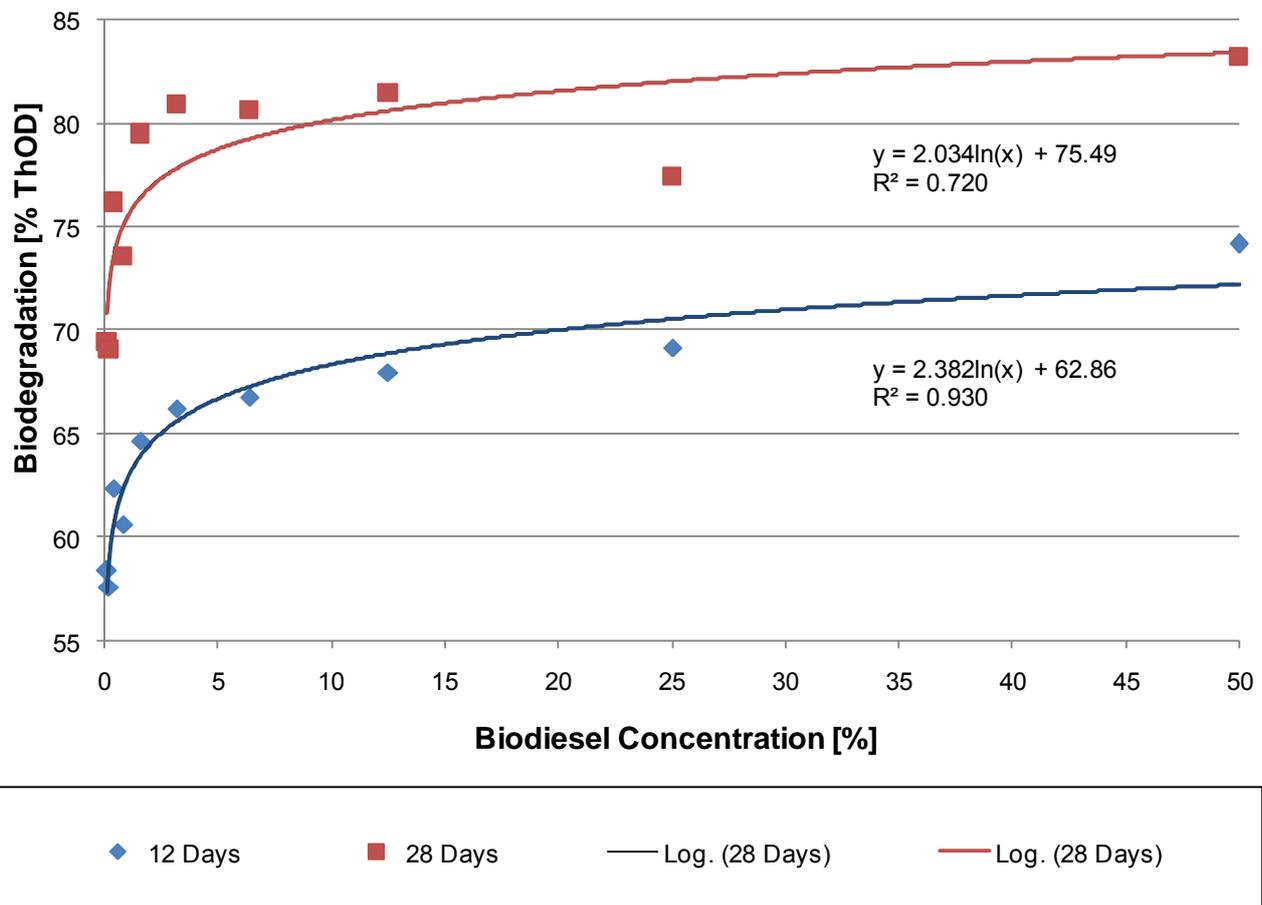


Figure 13: Regression analysis of the biodegradation rates of biodiesel/GTL-derived diesel blends.

The regression analysis showed that 0.28% soybean derived biodiesel is required in a biodiesel/GTL-derived diesel for it to pass the 10-day window “readily biodegradable” criteria according to the requirements of the Ready Biodegradability test (OECD, 301 F) using a manometric respirometer. Logarithmic regression was used because the biodiesel/GTL diesel ratio covered a wide range of values and when extrapolation to the lower values are performed, the predicted biodegradation values will not be less than zero, which would be inadmissible.

Biodiesel / GTL / EN590 blends were also tested and the results illustrated in Figure 14

and Table 12. Although biodegradation was rapid initially, it slowed down after approximately 11 days. None of the diesel blends achieved the 10-day window pass criteria and although the 30% biodiesel sample achieved 59.94% degradation. All the blends can be classified as inherently biodegradable since all samples achieved greater than 60% degradation by the end of the 28 day exposure period.

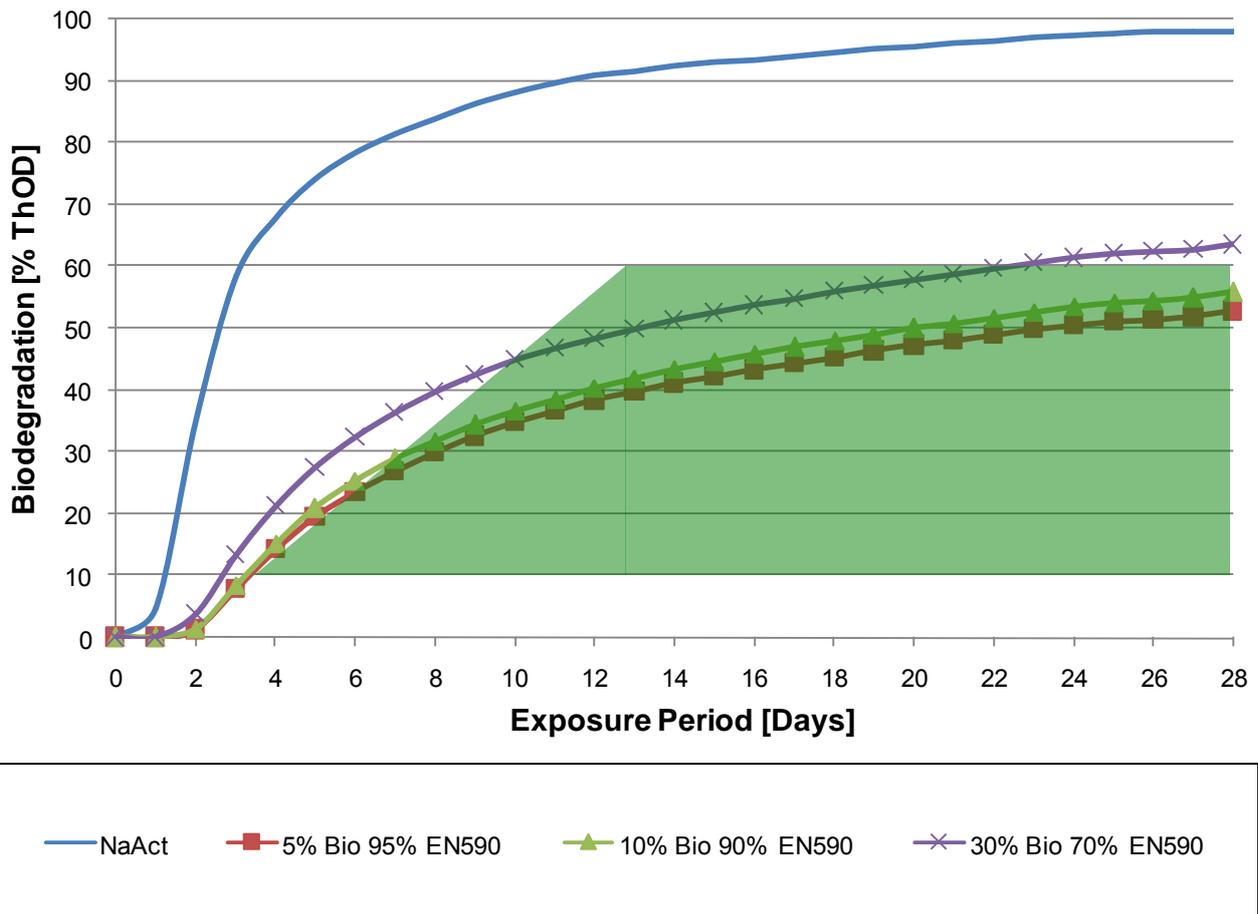


Figure 14: Biodegradation of biodiesel / GTL diesel / EN590 diesel blends by the respirometric “ready biodegradability” test.

Table 12: Biodegradation of Biodiesel / GTL Diesel / EN590 diesel blends at 12 and 28 days.

Biodiesel / EN590 diesel blends	Biodegradation [% ThOD ± StDev]	
	12 Days	28 Days
5% Biodiesel and 47.5% GTL diesel addition to EN590 diesel	49.40 ± 3.4	60.26 ± 2.8
10% Biodiesel and 45% GTL diesel addition to EN590 diesel	52.97 ± 4.5	60.85 ± 3.9
30% Biodiesel and 35% GTL diesel addition to EN590 diesel	59.94 ± 2.2	69.49 ± 1.2

3.4. Ecotoxicity Tests

3.4.1. Fish, Acute Toxicity Test

No fish mortality was observed after exposure to the WAFs of the different diesels and diesel blends. The fish ecotoxicity test on all diesel samples showed no toxicity at WAF loading rates of 1 000mg/L. This indicated that the concentration of dissolved diesel hydrocarbon constituents did not have an effect on the mortality of the fish. No fish toxicity tests were performed on biodiesel/GTL diesel blends because of the negative results found in the initial tests. Fish toxicity test results are presented in Table 13.

Table 13: Acute fish toxicity results from the fish that were exposed to the various WAFs of the diesels and diesel blending material.

Diesel sample	WAF Concentration	Exposed fish		Mortality	Mortality [%]
GTL 100% diesel	1 000	7	7	0	0
GTL 100% diesel	500	7	7	0	0
GTL 100% diesel	250	7	7	0	0
GTL 100% diesel	100	7	7	0	0
Natref Final Diesel 100%	1 000	7	7	0	0
Natref Final Diesel 100%	100	7	7	0	0
EN590 diesel	1 000	7	7	0	0
EN590 diesel	100	7	7	0	0
Biodiesel100 (Soybean derived fatty acid methyl esters (FAME))	1 000	7	7	0	0
Biodiesel100 (Soybean derived fatty acid methyl esters (FAME))	500	7	7	0	0
Biodiesel100 (Soybean derived fatty acid methyl esters (FAME))	250	7	7	0	0
Biodiesel100 (Soybean derived fatty acid methyl esters (FAME))	100	7	7	0	0
DHT Light Secunda diesel	1 000	7	7	0	0
DHT Light Secunda diesel	100	7	7	0	0
DSC Heavy Secunda diesel	1 000	7	7	0	0
DSC Heavy Secunda diesel	100	7	7	0	0
Creosote diesel blending component	1 000	7	7	0	0
Creosote diesel blending component	100	7	7	0	0
CatPoly diesel blending component	1 000	7	7	0	0
CatPoly diesel blending component	100	7	7	0	0
5% Biodiesel and 47.5% GTL diesel	1 000	7	7	0	0

addition to EN590 diesel					
10% Biodiesel and 45% GTL diesel addition to EN590 diesel	1 000	7	7	0	0
30% Biodiesel and 35% GTL diesel addition to EN590 diesel	1 000	7	7	0	0

3.4.2. *Daphnia magna*, Acute Immobilisation Test

No response was observed from control *D. magna*. *D. magna* response observed from the diesel 1 000mg/L WAF loading rates indicated that the biodiesel (B100) and Creosote diesel blending material showed an effect on the mobility of the crustaceans. The effect was below 20% of the individuals tested and subsequently the EC₅₀ concentration could not be calculated. The biodiesel (B100) showed 10% effect at a WAF loading rate of 500mg/L.

The biodiesel mixtures with GTL diesel did effect the mobility of the *D. magna* at 25% and 50% biodiesel addition at a WAF loading rate of 1 000mg/L. The biodiesel/GTL/EN590 diesel blends showed a response at biodiesel addition at 10% and 30% at WAF loading rates of 1 000mg/L. The response was marginal and in no instance did the *D. magna* show an effect on their mobility of 20% or more. No toxicity was observed in *D. magna* exposed to diesel WAF loading rates of 100mg/L. Results are presented in Table 14.

Table 14: Acute *D. magna* immobilisation test results from the exposure to the various WAFs of the diesels and diesel blending material.

Diesel sample	WAF Concentration	Effect [%]	
		Mean	StDev
GTL 100% diesel	1 000	0.0	0.0
GTL 100% diesel	500	0.0	0.0
GTL 100% diesel	250	0.0	0.0
GTL 100% diesel	100	0.0	0.0
Natref Final Diesel 100%	1 000	0.0	0.0
Sasol Natref Final Diesel 100%	100	0.0	0.0
EN590 diesel	1 000	0.0	0.0
EN590 diesel	100	0.0	0.0
Biodiesel100 (Soybean derived fatty acid methyl esters (FAME))	1 000	20.0	16.3
Biodiesel100 (Soybean derived fatty acid methyl esters (FAME))	500	10.0	11.5
Biodiesel100 (Soybean derived fatty acid	250	0.0	0.0

methyl esters (FAME))			
Biodiesel100 (Soybean derived fatty acid methyl esters (FAME))	100	0.0	0.0
DHT Light Secunda diesel	1 000	0.0	0.0
DHT Light Secunda diesel	100	0.0	0.0
DSC Heavy Secunda diesel	1 000	0.0	0.0
DSC Heavy Secunda diesel	100	0.0	0.0
Creosote diesel blending component	1 000	5.0	10.0
Creosote diesel blending component	100	0.0	0.0
CatPoly diesel blending component	1 000	0.0	0.0
CatPoly diesel blending component	100	0.0	0.0
5% Biodiesel addition to EN590 diesel	1 000	0.0	0.0
10% Biodiesel addition to EN590 diesel	1 000	0.0	0.0
30% Biodiesel addition to EN590 diesel	1 000	0.0	0.0
0.1%Biodiesel addition to GTL diesel	1 000	0.0	0.0
0.2% Biodiesel addition to GTL diesel	1 000	0.0	0.0
0.4% Biodiesel addition to GTL diesel	1 000	0.0	0.0
0.8% Biodiesel addition to GTL diesel	1 000	0.0	0.0
1.6% Biodiesel addition to GTL diesel	1 000	0.0	0.0
3.2% Biodiesel addition to GTL diesel	1 000	0.0	0.0
6.4% Biodiesel addition to GTL diesel	1 000	0.0	0.0
12.5% Biodiesel addition to GTL diesel	1 000	0.0	0.0
12.5% Biodiesel addition to GTL diesel	500	0.0	0.0
12.5% Biodiesel addition to GTL diesel	250	0.0	0.0
25% Biodiesel addition to GTL diesel	1 000	15.0	10.0
25% Biodiesel addition to GTL diesel	500	0.0	0.0
25% Biodiesel addition to GTL diesel	250	0.0	0.0
50% Biodiesel addition to GTL diesel	1 000	15.0	10.0
50% Biodiesel addition to GTL diesel	500	0.0	0.0
50% Biodiesel addition to GTL diesel	250		
5% Biodiesel and 47.5% GTL diesel addition to EN590 diesel	1 000	0.0	0.0
10% Biodiesel and 45% GTL diesel addition to EN590 diesel	1 000	5.0	10.0
30% Biodiesel and 35% GTL diesel addition to EN590 diesel	1 000	10.0	11.5

3.4.3. Algal Growth Inhibition Test

The response of the algae to the WAF loading rates of 1 000mg/L was marginal and all diesel WAFs showed an inhibitory response $\leq 10\%$. At 100mg/L WAF loading rates, the inhibitory effect on the algal growth was similar, with no inhibitory effect greater than 10%

being observed.

Detailed algal ecotoxicity tests of biodiesel/GTL mixtures at biodiesel concentrations in a geometric range from 0.8% to 12.5% (B0.8 to B12.5) showed a general trend that EC_{20} concentrations decreased with increased biodiesel concentration (Fig. 15).

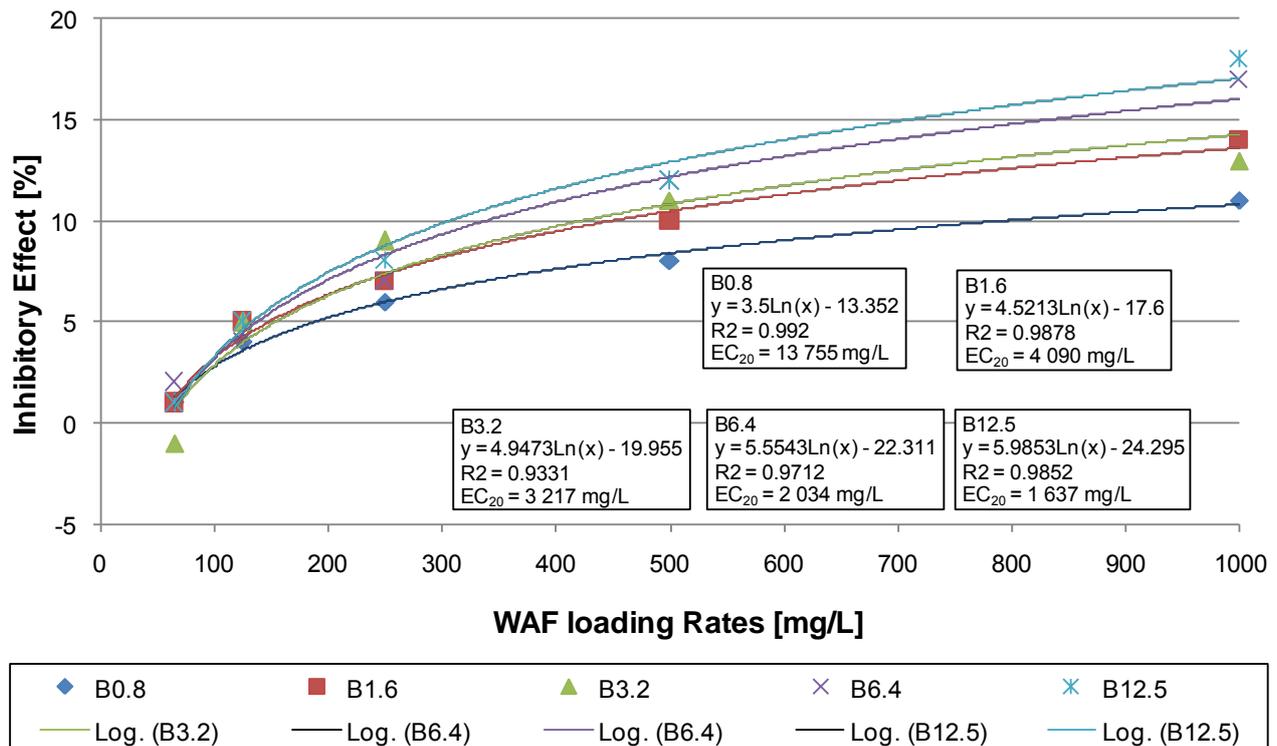


Figure 15: Logarithmic regression analysis of the ecotoxicity of the WAFs of biodiesel/GTL diesel blends at biodiesel concentrations with *Pseudokirchneriella subcapitata* in a geometric range from 0.8% to 12.5%.

3.4.4. BioTox™ *Vibrio fischeri* Bioluminescence Inhibition Test

The response of the marine bacteria, *Vibrio fischeri*, to the WAF loading rates of 1 000mg/L and 10% was marginal and all diesel WAFs showed an inhibitory response with the exception of GTL diesel. The inhibitory effect observed was not greater than 10% and results are presented in Figure 16.

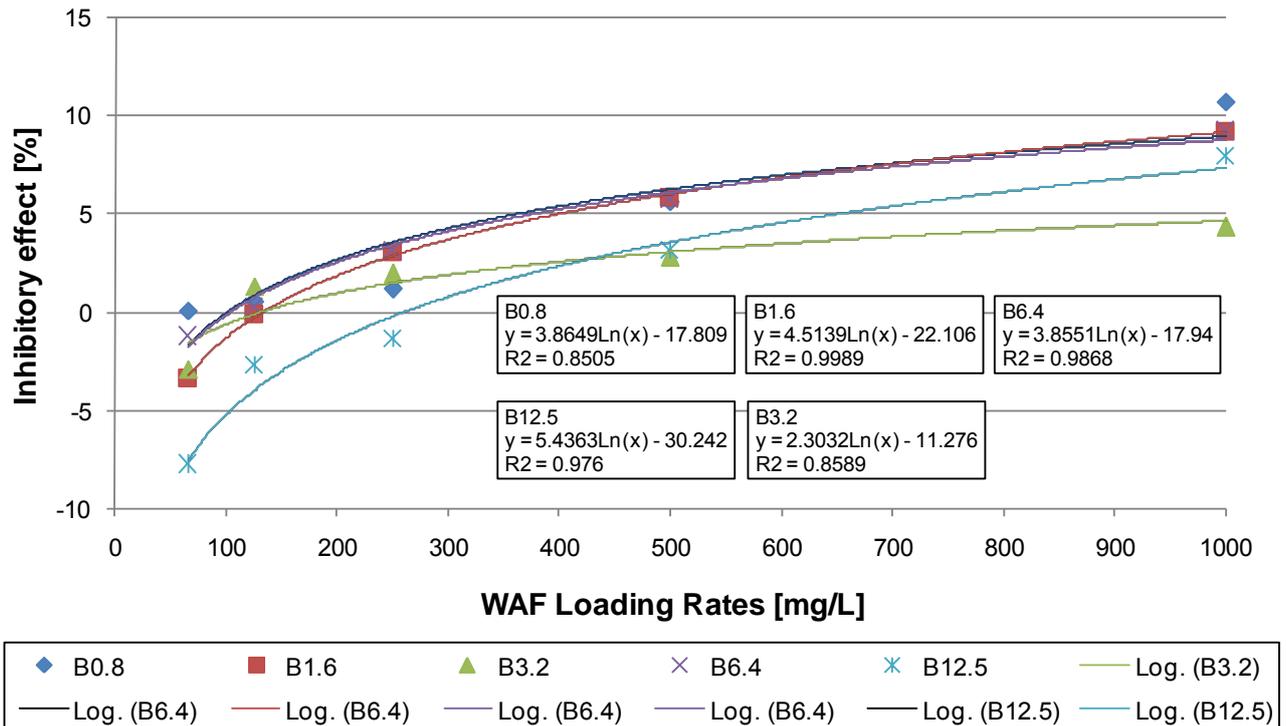


Figure 16: Logarithmic regression analysis of ecotoxicity of the WAFs of the biodiesel/GTL diesel blends at biodiesel concentrations with *V. fischeri* in a geometric range from 0.8% to 12.5%.

No trend was observed for bacterial ecotoxicity tests of biodiesel/GTL mixtures at biodiesel concentrations in a geometric range from 0.8% (B0.8) to 12.5% (B12.5) (Fig. 16). Overall ecotoxicity of the samples were below 15% and hence the EC₂₀ concentration could not be calculated. No inhibitory effect at 65mg/L WAF loading rates was observed for the biodiesel blends.

Table 15 presents a summary of the standard ecotoxicity tests that were performed on the WAFs of the diesels and diesel blends.

Table 15: A summary of the results from the ecotoxicity tests performed on the WAFs of the crude derived diesel, biodiesel and GTL diesel and CTL diesel blending material.

Diesel	Fish LC ₅₀	Crustacean EC ₅₀	Algae EC ₅₀	Bacteria EC ₅₀
GTL 100% diesel	> 1 000	> 1 000	< 100	> 100
Natref Final Diesel 100%	> 1 000	> 100	< 100	< 100
EN590 diesel	> 1 000	> 1 000	< 100	< 100
Biodiesel100 (Soybean derived fatty acid methyl esters (FAME))	> 1 000	< 100	< 100	< 100
DHT Light Secunda diesel	> 1 000	> 1 000	< 100	< 100
DSC Heavy Secunda diesel	> 1 000	> 1 000	< 100	< 100
Creosote diesel blending component	> 1 000	> 100	< 100	< 100
CatPoly diesel blending component	> 1 000	> 1 000	< 100	< 100
5% Biodiesel addition to EN590 diesel	> 1 000	> 1 000	< 100	< 100
10% Biodiesel addition to EN590 diesel	> 1 000	> 1 000	< 100	< 100
30% Biodiesel addition to EN590 diesel	> 1 000	> 100	< 100	< 100
0.1%Biodiesel addition to GTL diesel	NP	> 1 000	> 100	> 1 000
0.2% Biodiesel addition to GTL diesel	NP	> 1 000	> 100	> 1 000
0.4% Biodiesel addition to GTL diesel	NP	> 1 000	> 100	> 1 000
0.8% Biodiesel addition to GTL diesel	NP	> 1 000	> 100	> 1 000
1.6% Biodiesel addition to GTL diesel	NP	> 1 000	> 100	> 100
3.2% Biodiesel addition to GTL diesel	NP	> 1 000	> 100	< 100
6.4% Biodiesel addition to GTL diesel	NP	> 1 000	> 100	< 100
12.5% Biodiesel addition to GTL diesel	NP	> 1 000	> 100	< 100
25% Biodiesel addition to GTL diesel	NP	> 1 000	< 100	< 100
50% Biodiesel addition to GTL diesel	NP	> 1 000	< 100	< 100
5% Biodiesel and 47.5% GTL diesel addition to EN590 diesel	> 1 000	> 1 000	< 100	> 100
10% Biodiesel and 45% GTL diesel addition to EN590 diesel	> 1 000	> 1 000	< 100	> 100
30% Biodiesel and 35% GTL diesel addition to EN590 diesel	> 1 000	> 100	< 100	> 100

NP – Not performed

3.5. Quantitative structure-activity relationship

3.5.1. Ecotoxicity Estimation

The estimated exposure concentrations of the hydrocarbon concentrations expected to be present in the WAFs of the respective diesel blends are presented in Tables 16 and 17 for WAF loading rates of 100mg/L and 1 000mg/L respectively as calculated by the Fisk Ecotoxicity Estimation Model (FEEM). The model estimated that the crude derived diesel WAFs, Natref diesel and EN590 reference diesel, would contain in the highest estimated hydrocarbon load (Figures 15 and 16) for the 100mg/L and 1 000mg/L loading rates respectively. It was estimated that Natref diesel WAF contained a lower hydrocarbon concentration than the EN590 reference diesel.

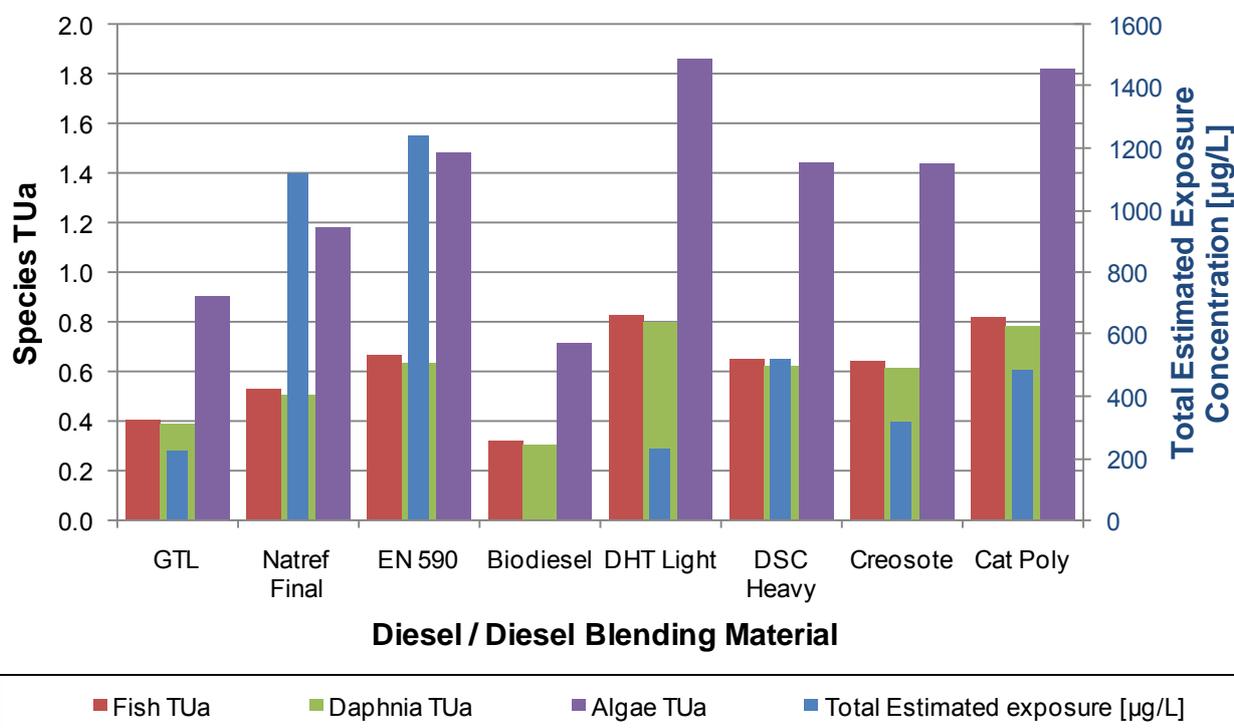


Figure 17: Estimated exposure concentrations and estimated species-specific acute toxicity units (TUa) values for the diesels and diesel blending material as calculated with the Fisk Ecotoxicity Estimation Model (FEEM) from 100mg/L loaded WAFs.

It was estimated that the paraffinic GTL diesel and DHT diesel blending material had the lowest total hydrocarbon concentrations followed by Creosote, CatPoly and DSC diesel blending materials. A similar trend was observed for the 1 000mg/L loaded WAFs, with marginally increased total estimated hydrocarbon concentrations for the diesels and diesel blending materials.

The crude derived diesels also showed a larger difference in the estimated hydrocarbon concentration between the different loaded WAFs. The 100mg/L loaded WAFs for Natref and EN590 diesel showed estimated total hydrocarbon concentrations of 1 118.02 and 1 240.06 µg/L respectively as opposed to the estimated 1 321.73 µg/L and 1 444.85 µg/L hydrocarbon concentration respectively of the 1 000mg/L loaded WAFs.

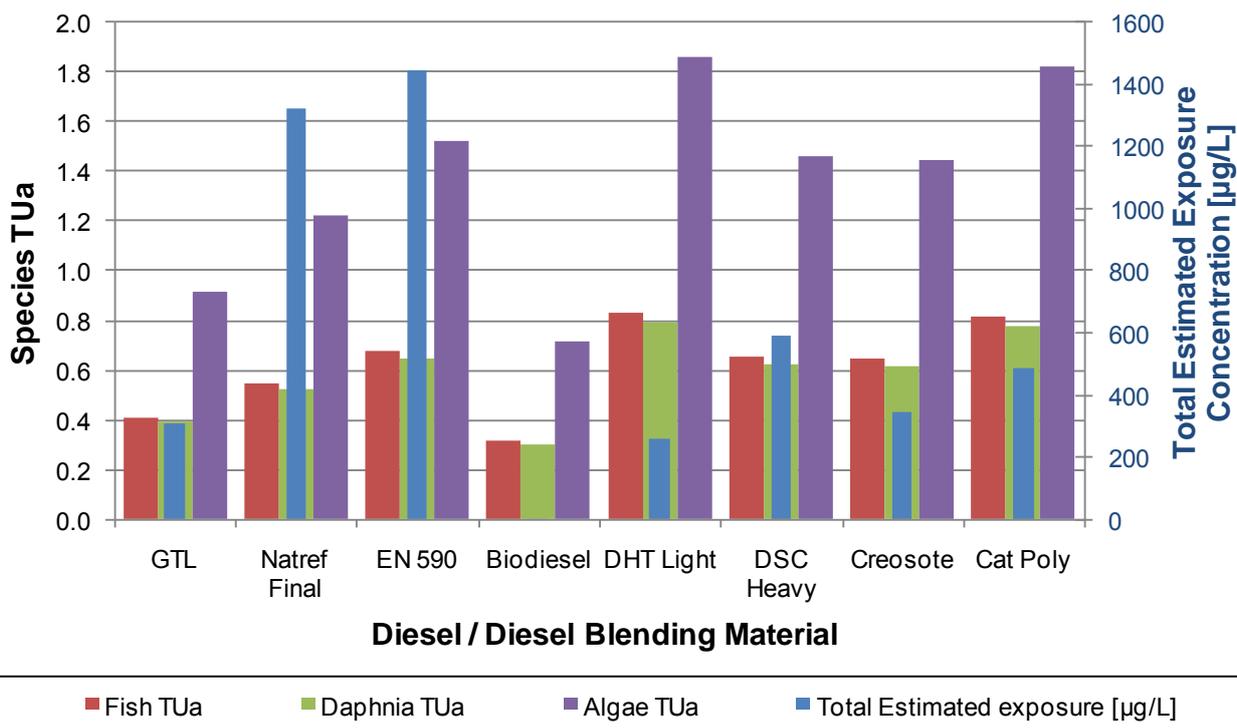


Figure 18: Estimated exposure concentrations and estimated species-specific acute toxicity units (TUa) values for the diesels and diesel blending material as calculated with the Fisk Ecotoxicity Estimation Model (FEEM) from 1 000mg/L loaded WAFs.

Table 16: Estimation of total hydrocarbon concentrations in the respective diesels and diesel blends at WAF loading rates of 100mg/L together with accompanying estimated acute toxicity units (TUa) concentrations.

Diesel	Estimated exposure [$\mu\text{g/L}$]	Acute Toxicity Unit (TUa)		
		Fish	Daphnia	Algae
GTL 100% diesel	230.84	0.40	0.39	0.90
Natref Final Diesel 100%	1 118.02	0.53	0.50	1.18
EN590 diesel	1 240.06	0.66	0.63	1.48
Biodiesel100 (Soybean derived fatty acid methyl esters (FAME))	0.45	0.32	0.30	0.71
DHT Light Secunda diesel	233.30	0.83	0.79	1.86
DSC Heavy Secunda diesel	517.46	0.65	0.62	1.45
Creosote diesel blending component	317.01	0.64	0.61	1.44
CatPoly diesel blending component	502.44	0.81	0.78	1.82
5% Biodiesel addition to EN590 diesel	1 178.077	0.43	0.39	0.90
10% Biodiesel addition to EN590 diesel	1 116.097	0.43	0.39	0.90
30% Biodiesel addition to EN590 diesel	868.174	0.43	0.39	0.90
0.1%Biodiesel addition to GTL diesel	230.61	0.43	0.39	0.90
0.2% Biodiesel addition to GTL diesel	230.380	0.43	0.38	0.90
0.4% Biodiesel addition to GTL diesel	229.919	0.43	0.38	0.90
0.8% Biodiesel addition to GTL diesel	228.998	0.42	0.38	0.89
1.6% Biodiesel addition to GTL diesel	227.155	0.41	0.38	0.88
3.2% Biodiesel addition to GTL diesel	223.468	0.40	0.37	0.86
6.4% Biodiesel addition to GTL diesel	216.096	0.36	0.34	0.81
12.5% Biodiesel addition to GTL diesel	202.042	0.64	0.61	1.44
25% Biodiesel addition to GTL diesel	173.242	0.62	0.60	1.40
50% Biodiesel addition to GTL diesel	115.644	0.55	0.53	1.25
5% Biodiesel and 47.5% GTL diesel addition to EN590 diesel	698.699	0.53	0.50	1.17
10% Biodiesel and 45% GTL diesel addition to EN590 diesel	661.949	0.52	0.49	1.14
30% Biodiesel and 35% GTL diesel addition to EN590 diesel	514.948	0.47	0.45	1.05

Table 17: Estimation of total hydrocarbon concentrations in the respective diesels and diesel blends at WAF loading rates of 1 000mg/L together with accompanying estimated acute toxicity units (TUa) concentrations.

Diesel	Estimated exposure [$\mu\text{g/L}$]	Acute Toxicity Unit (TUa)		
		Fish	Daphnia	Algae
GTL 100% diesel	310.05	0.41	0.39	0.92
Natref Final Diesel 100%	1 321.73	0.55	0.52	1.22
EN590 diesel	1 444.85	0.68	0.65	1.52
Biodiesel100 (Soybean derived fatty acid methyl esters (FAME))	0.48	0.32	0.30	0.71
DHT Light Secunda diesel	256.16	0.83	0.80	1.86
DSC Heavy Secunda diesel	588.49	0.65	0.62	1.46
Creosote diesel blending component	346.09	0.65	0.62	1.45
CatPoly diesel blending component	802.52	0.81	0.78	1.82
5% Biodiesel addition to EN590 diesel	1 372.63	0.41	0.39	0.91
10% Biodiesel addition to EN590 diesel	1 300.42	0.41	0.39	0.91
30% Biodiesel addition to EN590 diesel	1 011.54	0.41	0.39	0.91
0.1%Biodiesel addition to GTL diesel	309.74	0.41	0.39	0.91
0.2% Biodiesel addition to GTL diesel	309.43	0.41	0.39	0.91
0.4% Biodiesel addition to GTL diesel	308.81	0.41	0.39	0.91
0.8% Biodiesel addition to GTL diesel	307.57	0.40	0.38	0.90
1.6% Biodiesel addition to GTL diesel	305.09	0.40	0.38	0.89
3.2% Biodiesel addition to GTL diesel	300.14	0.39	0.37	0.86
6.4% Biodiesel addition to GTL diesel	290.23	0.36	0.35	0.81
12.5% Biodiesel addition to GTL diesel	271.35	0.66	0.63	1.48
25% Biodiesel addition to GTL diesel	232.65	0.64	0.62	1.44
50% Biodiesel addition to GTL diesel	155.26	0.57	0.55	1.28
5% Biodiesel and 47.5% GTL diesel addition to EN590 diesel	833.60	0.53	0.51	1.19
10% Biodiesel and 45% GTL diesel addition to EN590 diesel	789.75	0.52	0.50	1.17
30% Biodiesel and 35% GTL diesel addition to EN590 diesel	614.36	0.48	0.46	1.07

The predicted TUa contribution of the paraffin and aromatics of the 100mg/L and 1 000mg/L WAF loading rates of the diesel and diesel blending materials were calculated and are presented in Figures 19 – 24. In all estimations the combined contribution of the estimated toxicity was attributed to the paraffin in the diesel.

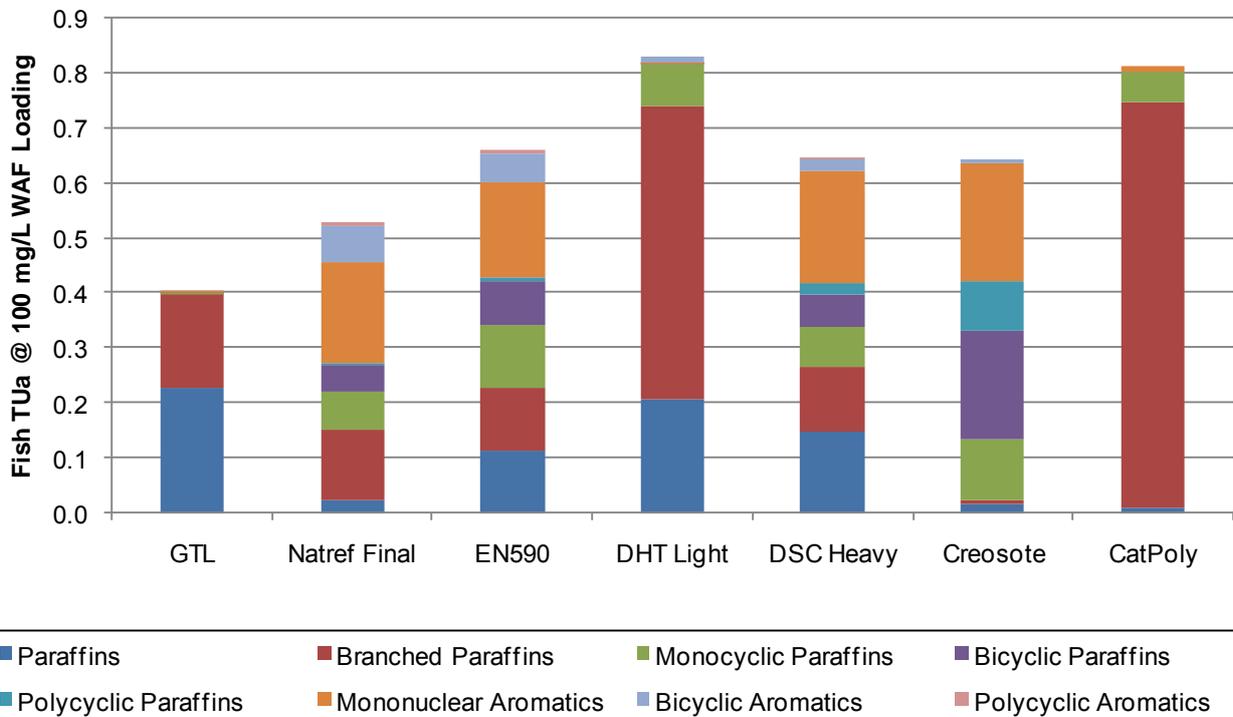


Figure 19: Acute Toxicity Unit (TUa) contribution of paraffin and aromatic compounds for fish at 100mg/L WAF loading rates for the respective diesels and diesel blending materials using the FEEM.

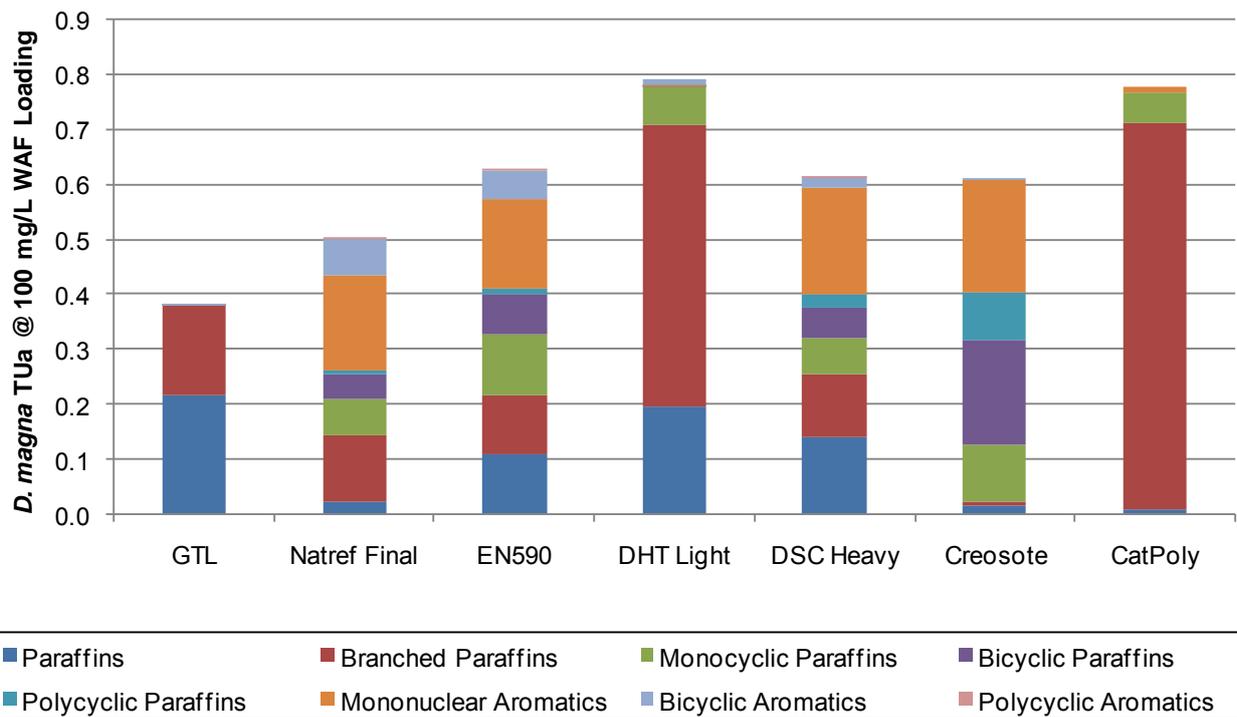


Figure 20: Acute Toxicity Unit (TUa) contribution of paraffin and aromatic compounds for *Daphnia* at 100mg/L WAF loading rates for the respective diesels and diesel blending materials using the FEEM.

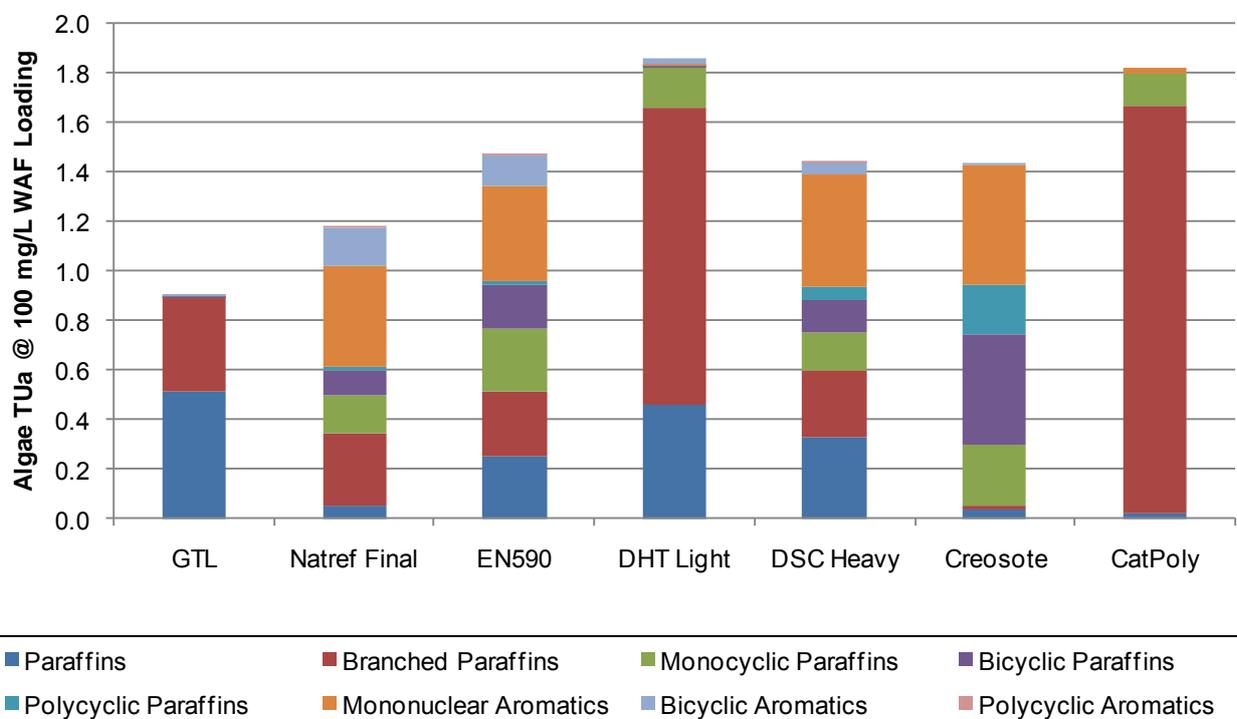


Figure 21: Acute Toxicity Unit (TUa) contribution of paraffin and aromatic compounds for algae at 100mg/L WAF loading rates for the respective diesels and diesel blending materials using the FEEM.

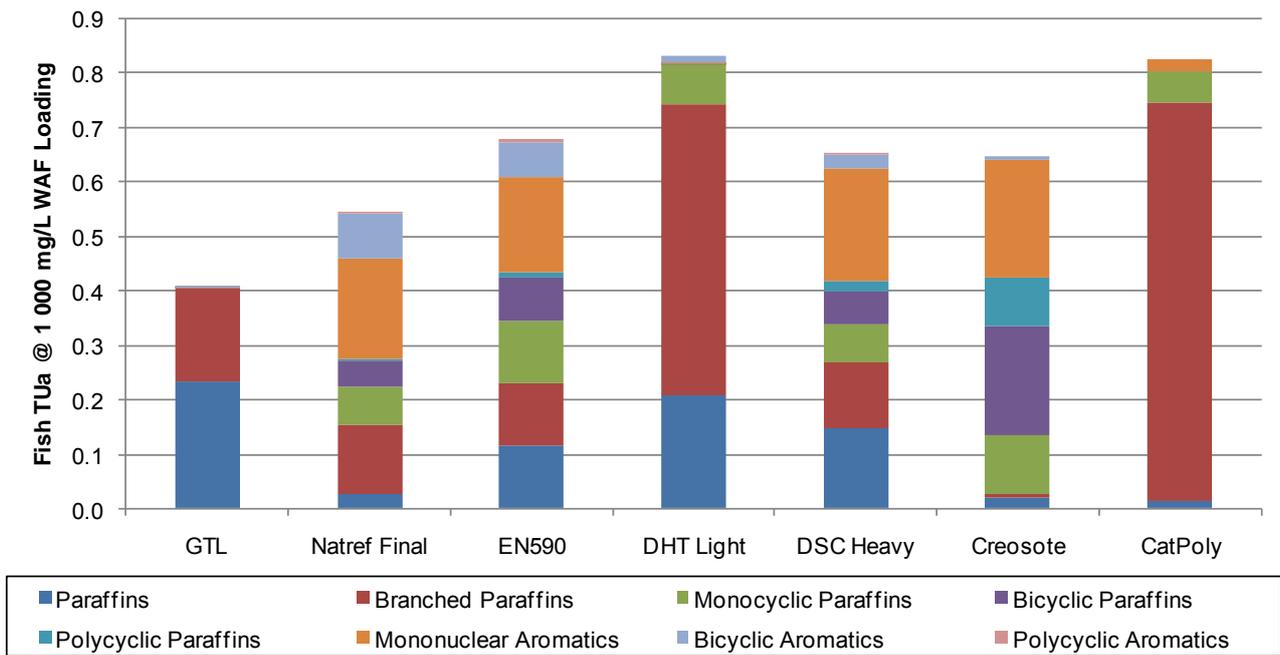


Figure 22: Acute Toxicity Unit (TUa) contribution of paraffin and aromatic compounds for fish at 1 000mg/L WAF loading rates for the respective diesels and diesel blending materials using the FEEM.

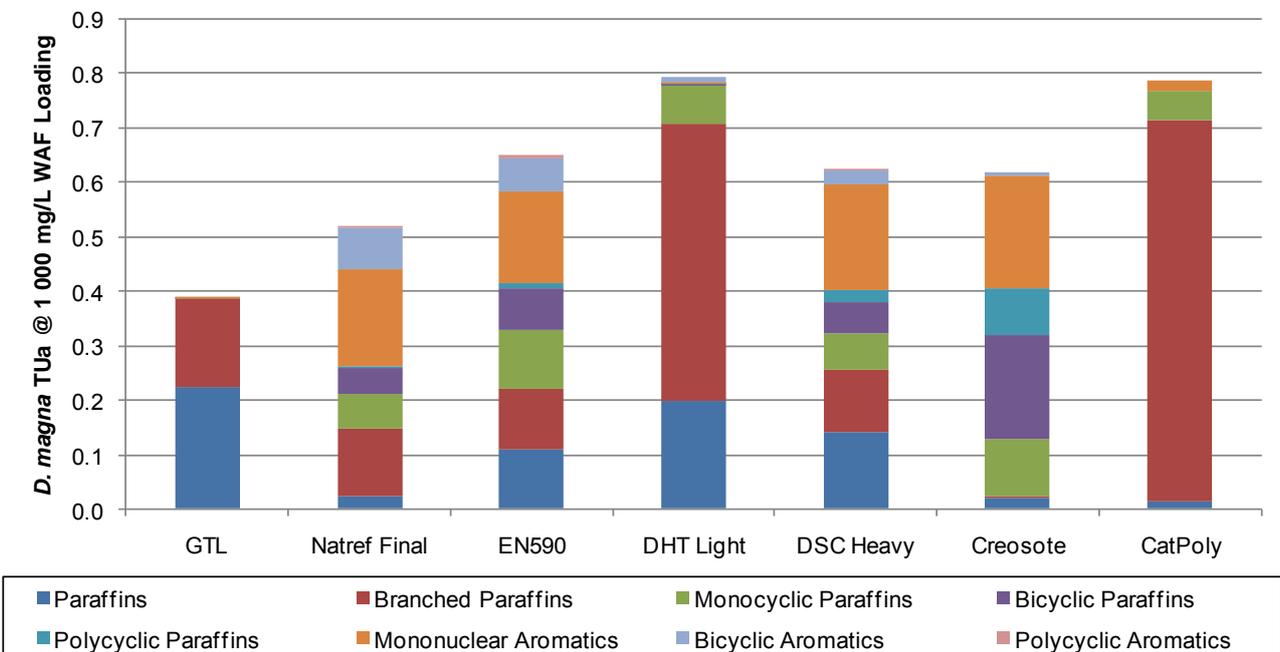


Figure 23: Acute Toxicity Unit (TUa) contribution of paraffin and aromatic compounds for *Daphnia* at 1 000mg/L WAF loading rates for the respective diesels and diesel blending materials using the FEEM.

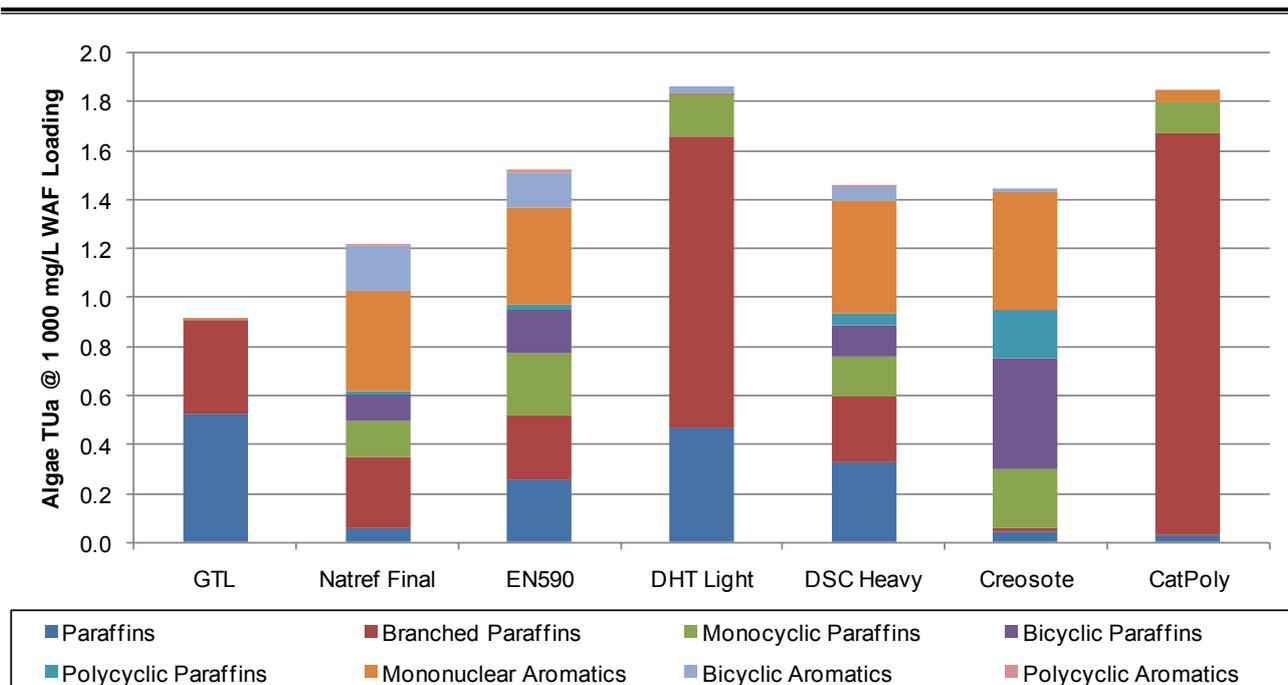


Figure 24: Acute Toxicity Unit (TUa) contribution of paraffin and aromatic compounds for algae at 1 000mg/L WAF loading rates for the respective diesels and diesel blending materials using the FEEM.

The predicted toxicity, as calculated by the Fisk Estimated Ecotoxicity Model (FEEM) showed that the algae would be the most affected by the WAF hydrocarbons by having the highest estimated TUa values and the *Daphnia sp.* and fish the least affected by having the lowest estimated TUa values. The biodiesel showed the lowest TUa values for all taxa. According to the FEEM model, Fisk (2005), a TUa value of greater than 1 unit would indicate the point where the theoretical WAF could potentially be toxic to the taxa. Both crude derived diesels, together with the four CTL diesel blending materials (DHT, DSC, Creosote and CatPoly) showed algal TUa values greater than 1, which indicates potential toxicity to algae from exposure to the WAFs.

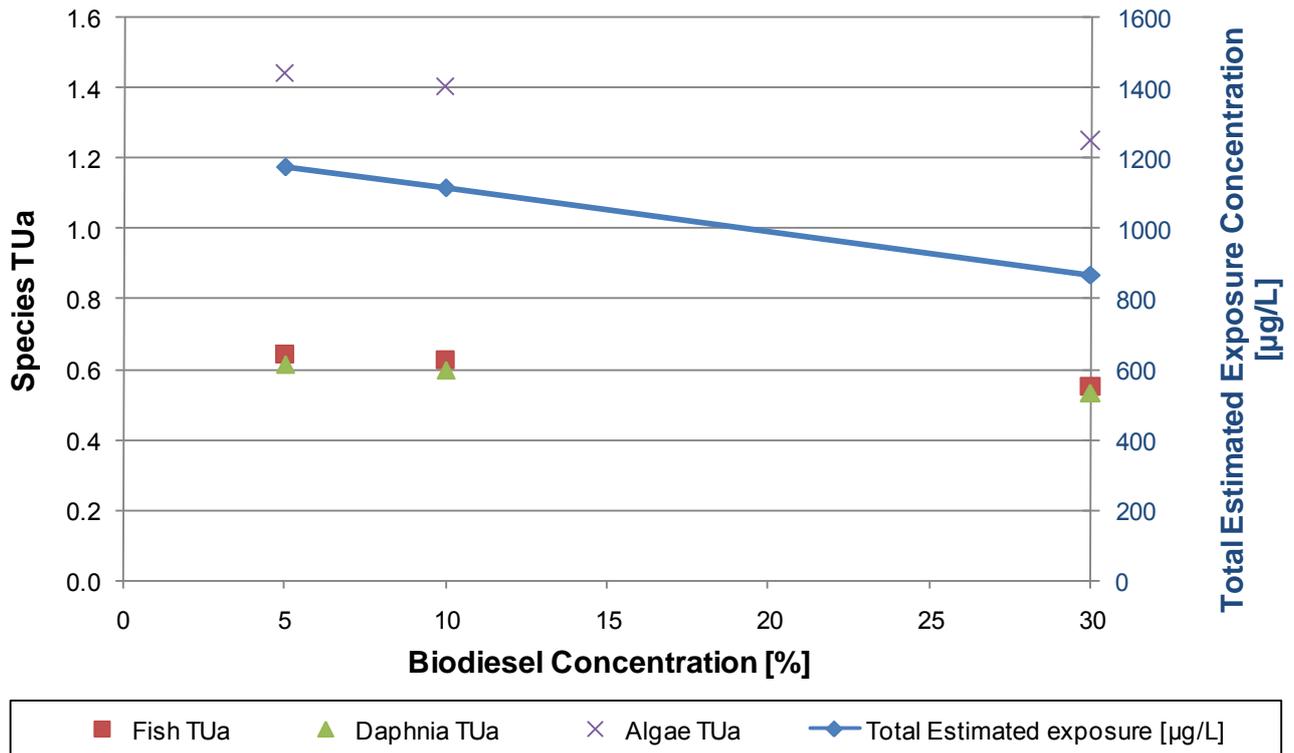


Figure 25: Estimated exposure concentrations and estimated species-specific acute toxicity units (TUa) values for the biodiesel / EN590 diesel experimental blends as calculated with the Fisk Ecotoxicity Estimation Model (FEEM) from 100mg/L loaded WAFs.

The estimated total hydrocarbon concentration and estimated species-specific toxicity units (TUa) values for the biodiesel / EN590 diesel experimental blends are presented in Figures 25 and 26 for the 100mg/L and 1 000mg/L loaded WAFs respectively. The total estimated hydrocarbon concentration in the WAFs showed a decrease with increasing biodiesel concentration. The addition of biodiesel reduced the concentration of hydrocarbons in the WAFs which in turn resulted in the TUa values decreasing with an increase in biodiesel concentration.

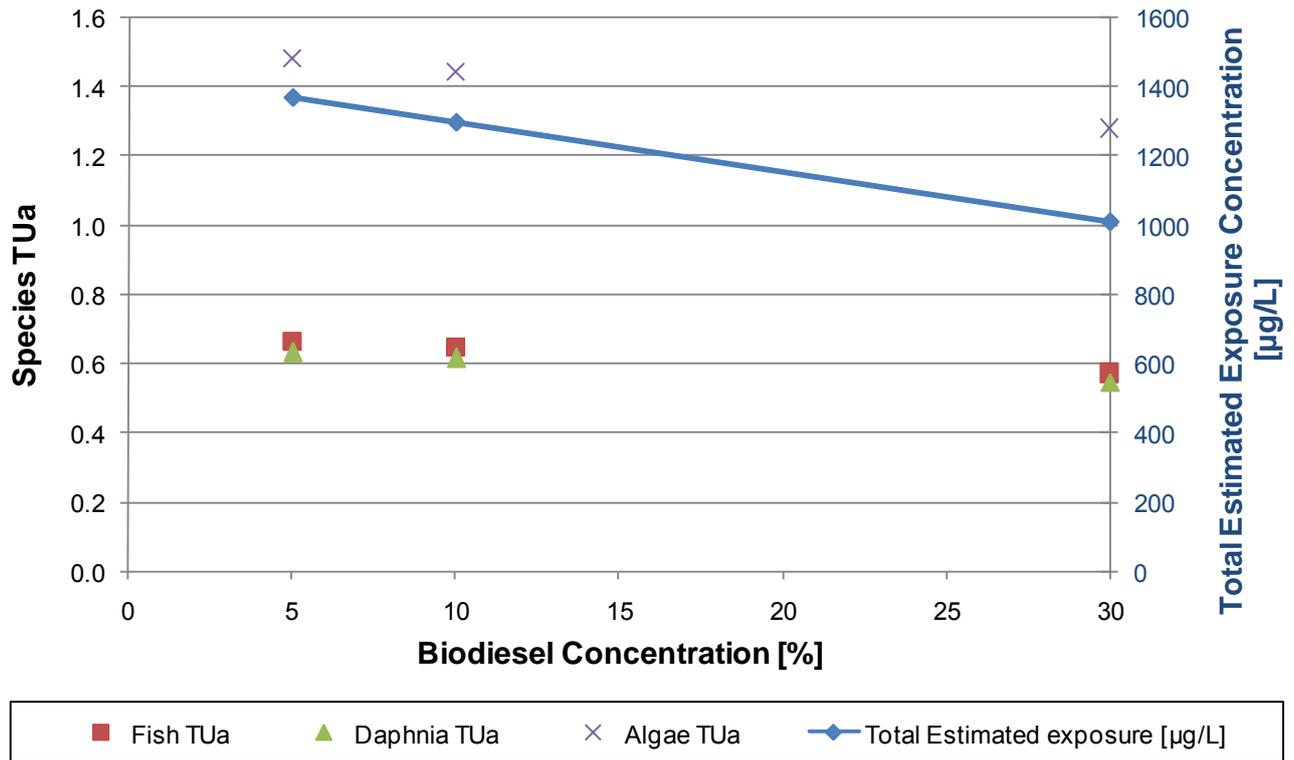


Figure 26: Estimated exposure concentrations and estimated species-specific acute toxicity units (TUa) values for the biodiesel / EN590 diesel experimental blends as calculated with the Fisk Ecotoxicity Estimation Model (FEEM) from 1 000mg/L loaded WAFs.

The estimated TUa values for the biodiesel / EN590 diesel blends indicated that the algal TUa values were the highest with the TUa decreasing with increasing biodiesel concentration.

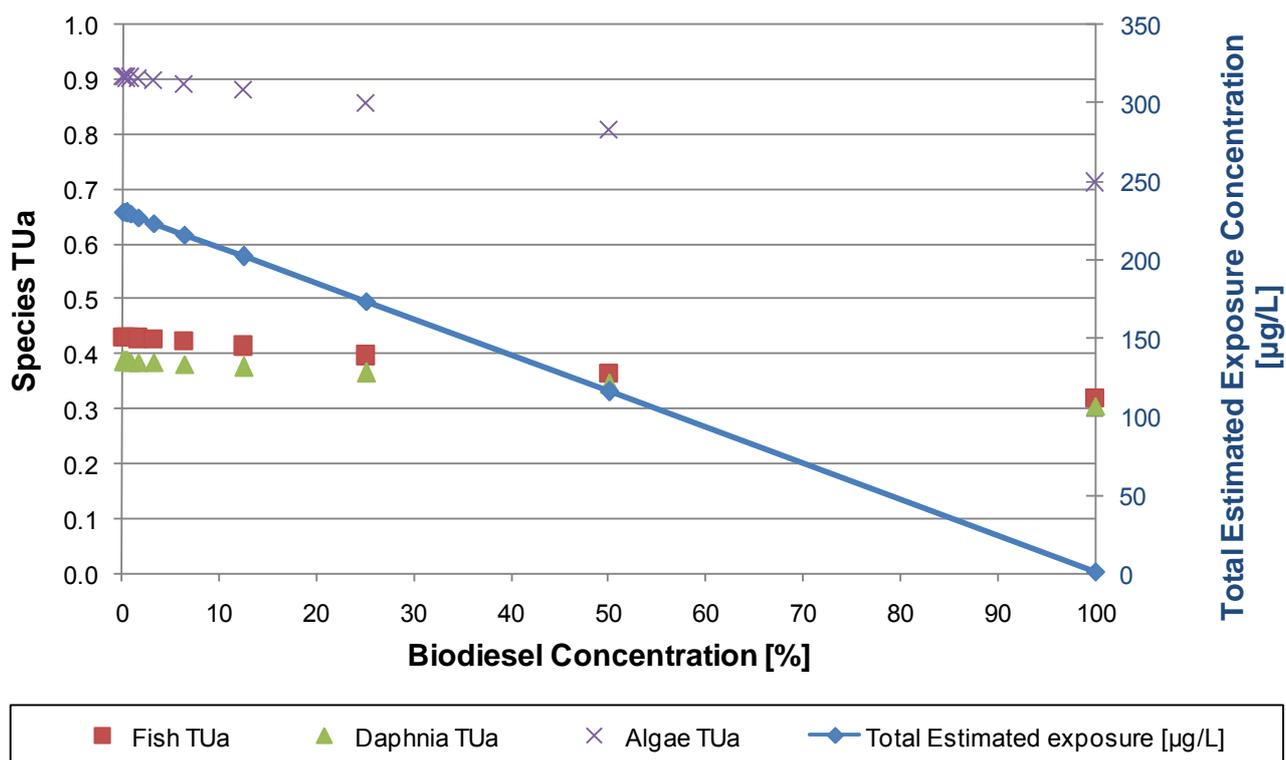


Figure 27: Estimated exposure concentrations and estimated species-specific acute toxicity units (TUa) values for the biodiesel / GTL diesel experimental blends as calculated with the Fisk Ecotoxicity Estimation Model (FEEM) from 100mg/L loaded WAFs.

The estimation of the hydrocarbon concentration contained in the theoretical WAFs of the biodiesel / GTL diesel experimental blends showed a decrease with increasing biodiesel concentration (Figures 27 and 28). With the GTL diesel having the lowest predicted hydrocarbon concentration of the petroleum diesels, it was estimated that the addition of biodiesel reduced the hydrocarbon concentration in the theoretical WAFs even more.

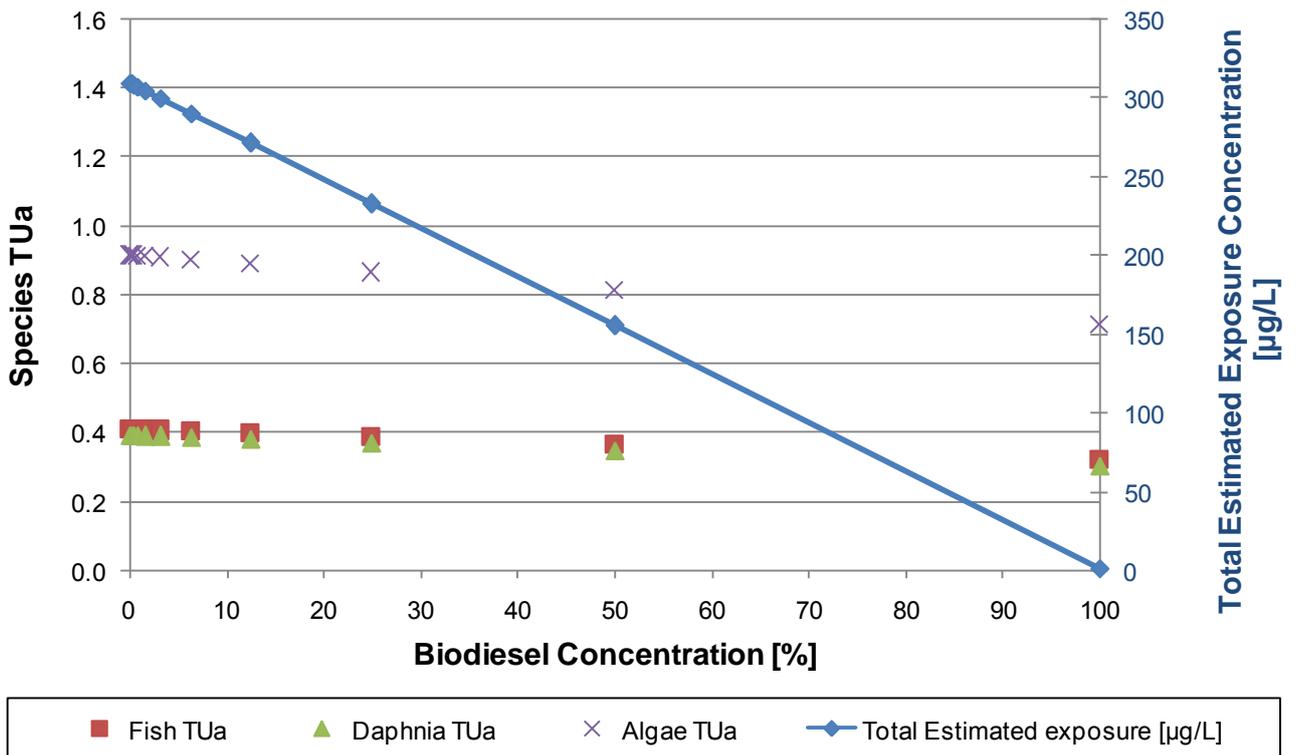


Figure 28: Estimated exposure concentrations and estimated species-specific acute toxicity units (TUa) values for the biodiesel / GTL diesel experimental blends as calculated with the Fisk Ecotoxicity Estimation Model (FEEM) from 1 000mg/L loaded WAFs.

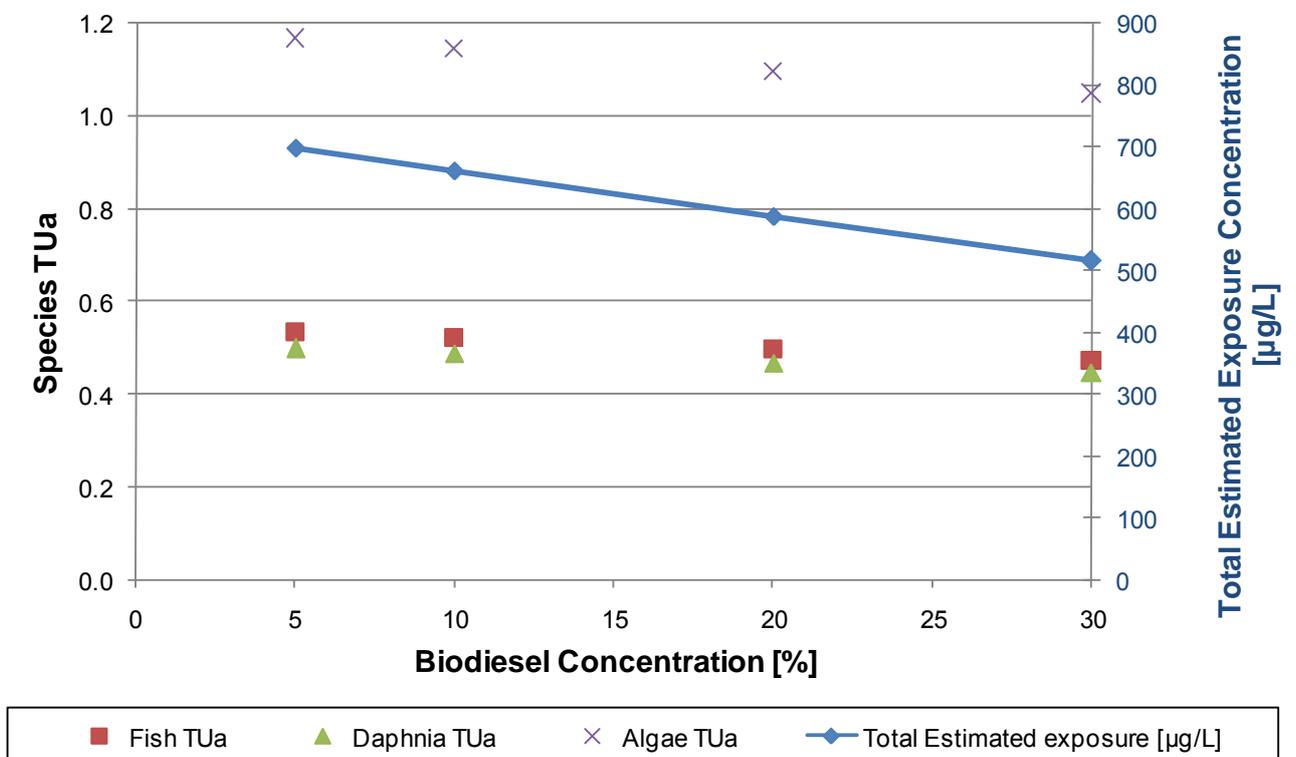


Figure 29: Estimated exposure concentrations and estimated species-specific acute toxicity units (TUa) values for the biodiesel / GTL diesel / EN590 experimental blends as

calculated with the Fisk Ecotoxicity Estimation Model (FEEM) from 100mg/L loaded WAFs.

The estimation of the hydrocarbon concentration contained in the WAFs of the biodiesel / GTL diesel / EN590 experimental blends showed a decrease with increasing biodiesel concentration (Figures 29 and 30). The predicted hydrocarbon concentration was lower when compared to the biodiesel / EN590 blends.

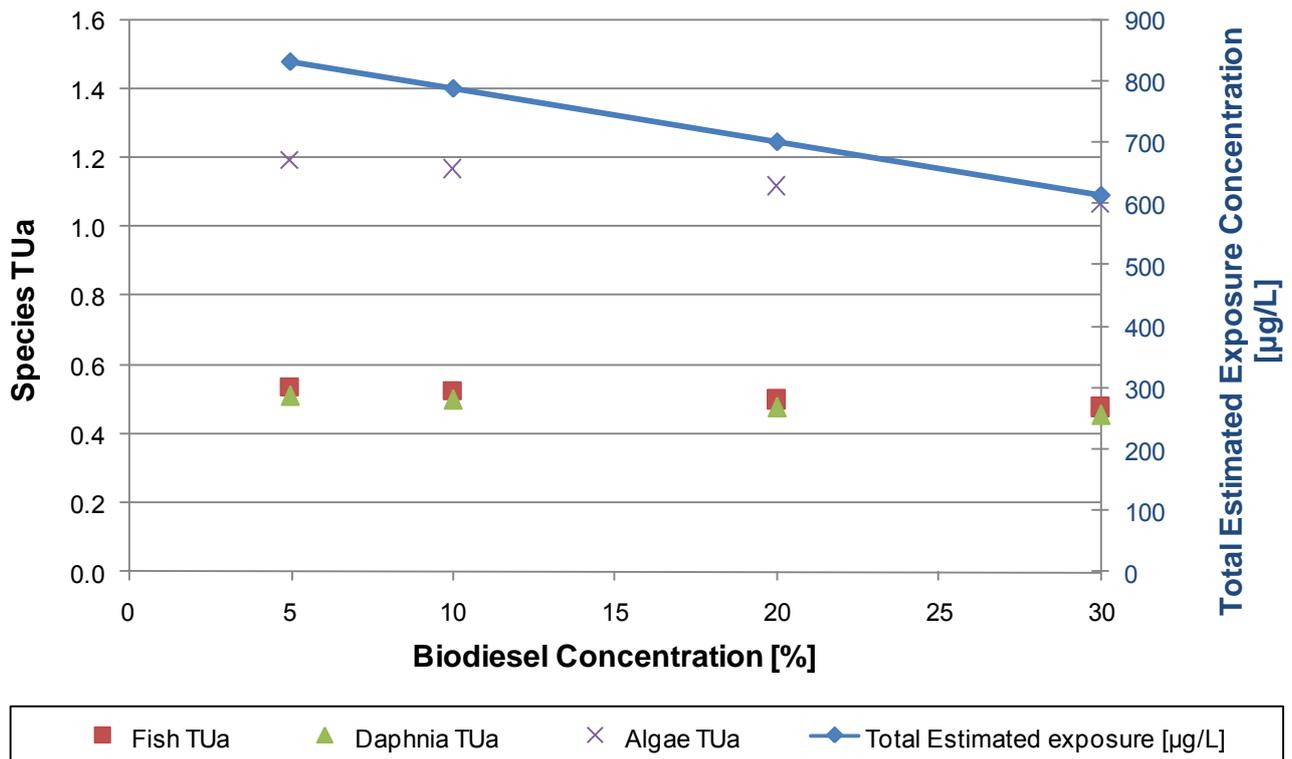


Figure 30: Estimated exposure concentrations and estimated species-specific acute toxicity units (TUa) values for the biodiesel / GTL diesel / EN590 experimental blends as calculated with the Fisk Ecotoxicity Estimation Model (FEEM) from 1 000mg/L loaded WAFs.

The estimated effect, as a measure of the TUa, of the biodiesel / GTL diesel / EN590 diesel experimental blends showed that WAFs at loading rates of up to 1 000mg/L would not be acutely toxic to all three taxa.

3.5.2. Biodegradability Estimation

The biodegradation estimations of the diesel and diesel blends using the Episuite BioWin 3 (ver. 4.1) and FEEM models are presented in Table 18. The estimation model for biodegradability potential corresponded with eight experimental biodegradability rates:

- Sasol GTL 100% diesel
- Sasol Natref Final Diesel 100%
- EN590 diesel
- DHT Light Secunda diesel
- 5% Biodiesel addition to EN590 diesel
- 10% Biodiesel addition to EN590 diesel
- 0.1% Biodiesel addition to GTL diesel
- 0.2% Biodiesel addition to GTL diesel

The model predictions and experimental biodegradability rates corresponded to all the complete diesels, with the exception of DCS Heavy Secunda diesel and Biodiesel 100.

The estimation model for biodegradability potential overestimated two experimental biodegradability rates:

- Creosote diesel blending component
- CatPoly diesel blending component

The two CTL diesel blending components' biodegradability potential was overestimated by the estimation model. The three biodiesel/EN590 diesel blends were also overestimated.

The estimation model for biodegradability potential underestimated 14 experimental biodegradability rates:

- Biodiesel100 (Soybean derived FAME)
- DSC Heavy Secunda diesel
- 30% Biodiesel addition to EN590 diesel
- 0.4% Biodiesel addition to GTL diesel
- 0.8% Biodiesel addition to GTL diesel
- 1.6% Biodiesel addition to GTL diesel
- 3.2% Biodiesel addition to GTL diesel

- 6.4% Biodiesel addition to GTL diesel
- 12.5% Biodiesel addition to GTL diesel
- 25% Biodiesel addition to GTL diesel
- 50% Biodiesel addition to GTL diesel
- 5% Biodiesel, 47.5% GTL diesel, EN590 diesel
- 10% Biodiesel, 45% GTL diesel, EN590 diesel
- 30% Biodiesel, 35% GTL diesel, EN590 diesel

Table 18: Biodegradation estimation results and experimental biodegradability rates of the diesels and diesel blends. The estimation results (Est. Result) indicate whether the predicted values Corresponded (C), Underestimated (U) or Overestimated (O) the experimental values.

Diesel	BioWin 3 Estimate	Biodegradability result	Exp. Biodegradability [%]		Biodegradability result	Est. Result
			12 Days	28 Days		
Sasol GTL 100% diesel	3.15	RBN	57.86 ± 0.90	76.7 ± 0.90	RBN	C
Sasol Natref Final Diesel 100%	2.66	IBN	28.86 ± 2.65	43.92 ± 1.56	IBN	C
EN590 diesel	2.15	IBN	36.52 ± 0.61	53.57 ± 1.61	IBN	C
Biodiesel100 (Soybean derived FAME)	2.99	IB	82.64 ± 2.74	97.85 ± 1.98	RB	U
DHT Light Secunda diesel	3.17	RBN	57.26 ± 3.83	70.59 ± 3.32	RBN	C
DSC Heavy Secunda diesel	2.61	IBN	54.11 ± 7.83	66.32 ± 5.01	IB	U
Creosote diesel blending component	2.81	IB	31.91 ± 3.28	45.37 ± 1.76	IBN	O
CatPoly diesel blending component	3.13	RBN	20.06 ± 0.76	34.74 ± 2.07	IBN	O
5% Biodiesel addition to EN590 diesel	2.19	IBN	38.43 ± 1.3	52.82 ± 1.9	IBN	C
10% Biodiesel addition to EN590 diesel	2.23	IBN	40.37 ± 2.3	55.88 ± 3.6	IBN	C
30% Biodiesel addition to EN590 diesel	2.40	IBN	48.43 ± 1.8	63.53 ± 3.2	IB	U
0.1%Biodiesel addition to GTL diesel	3.15	RBN	58.39 ± 1.4	69.43 ± 4.2	RBN	C
0.2% Biodiesel addition to GTL diesel	3.15	RBN	57.61 ± 4.8	69.08 ± 4.1	RBN	C
0.4% Biodiesel addition to GTL diesel	3.15	RBN	62.35 ± 6.3	76.20 ± 3.9	RB	U
0.8% Biodiesel addition to GTL	3.15	RBN	60.60 ± 2.7	73.55 ± 7.7	RB	U

diesel							
1.6% Biodiesel addition to GTL diesel	3.15	RBN	64.68 ± 3.6	79.48 ± 2.1	RB	U	
3.2% Biodiesel addition to GTL diesel	3.14	RBN	66.20 ± 5.4	80.90 ± 3.6	RB	U	
6.4% Biodiesel addition to GTL diesel	3.14	RBN	66.77 ± 3.0	80.64 ± 4.1	RB	U	
12.5% Biodiesel addition to GTL diesel	3.13	RBN	68.00 ± 2.5	81.46 ± 4.0	RB	U	
25% Biodiesel addition to GTL diesel	3.11	RBN	69.16 ± 2.1	77.42 ± 2.5	RB	U	
50% Biodiesel addition to GTL diesel	3.07	RBN	74.18 ± 4.4	83.21 ± 3.2	RB	U	
5% Biodiesel, 47.5% GTL diesel, EN590 diesel	2.67	IBN	49.40 ± 3.4	60.26 ± 2.8	IB	U	
10% Biodiesel, 45% GTL diesel, EN590 diesel	2.68	IBN	52.97 ± 4.5	60.85 ± 3.9	IB	U	
30% Biodiesel, 35% GTL diesel, EN590 diesel	2.72	IBN	59.94 ± 2.2	69.49 ± 1.2	RB	U	

Table 19: Biodegradability estimation results illustrating the ratio of the biodegradability result category in relation to the individual constituents found the diesels.

Biodegradability result	GTL	Natref Final	EN590	Biodiesel	DHT Light	DSC Heavy	Creosote	CatPoly
RB	28.79	4.16	15.19		22.41	19.94	6.88	1.30
RBN	38.30	39.06	35.42	11.00	66.72	36.79	27.21	90.92
IB	26.53	31.70	32.61	89.00	7.27	28.99	38.08	6.41
IBN	6.38	25.08	16.78		3.61	14.27	27.84	1.38

The results of the biodegradability estimation method showed that the GTL diesel comprised of approximately 28.8% ready biodegradable passing the 10-day window (RB) constituents, 38.3% read biodegradable not passing the 10-day window (RBN) constituents, 26.5% inherently biodegradable meeting the criteria (IB) constituents and 6.4% inherently biodegradable not meeting the criteria (IBN) constituents. Table 19 and Figure 31 shows the biodegradability fractions of the complete diesels and two diesel blending materials.

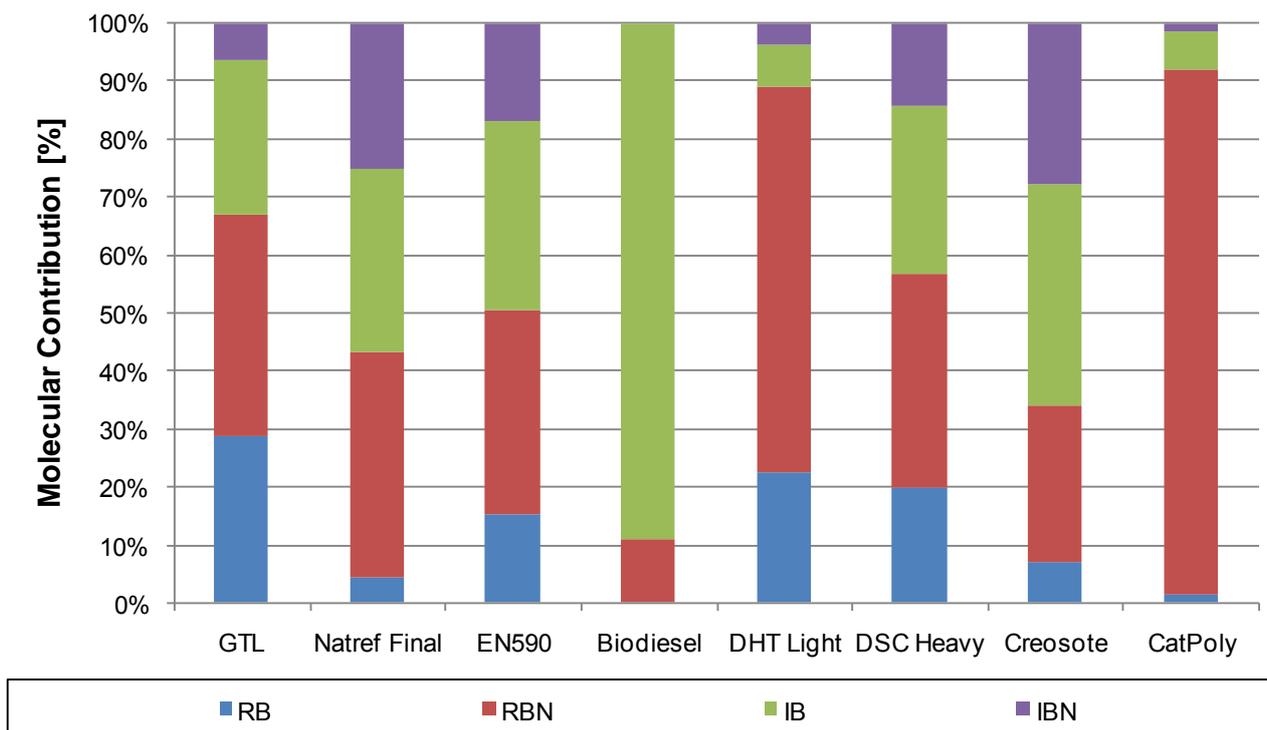


Figure 31: Graphical illustration of the biodegradability fractions of the complete diesels and two diesel blending materials according to the estimations of the BioWin 3 Ultimate biodegradation model and the FEEM model classes.

Discussion

This research aimed to evaluate whether the composition of diesels derived from different feed stocks, that included coal, natural gas, crude oil and soybean oil, would influence its biodegradability and ecotoxicity. The effect of blending biodiesel (derived from soybean oil) with GTL- and crude-oil derived diesel on biodegradation and ecotoxicity was also investigated. The manometric respirometric biodegradability test was used to assess the biodegradation rates of the various diesels and diesel blends. The results indicated that the biodiesel and GTL-derived diesel had a higher biodegradation rate than the crude oil-derived diesels. It also showed that biodiesel addition could increase biodegradability of GTL, CTL and crude-derived diesel blends. The ecotoxicity was measured using a battery of standard ecotoxicity tests with bacteria, algae, crustaceans and fish as sentinel organisms. The ecotoxicity tests indicated that the WAFs of the petroleum diesels had little or no acute toxic effect on the sentinel species. Slight toxicity was observed in the samples that contained biodiesel. The present study also assessed the applicability and accuracy of QSAR derived ecotoxicity and biodegradability estimations for the diesels and diesel blends that were tested. The results showed that QSAR derived estimations produced by the EpiSuite models were accurate for the petroleum diesel constituents, but not for the FAME found in biodiesel. When combined with the FEEM to generate ecotoxicity information and biodegradability estimations, the results were variable.

4.1. Fuel Composition

Accurate characterisation of petroleum fuels is important from the perspective of a producer in order to manage operating conditions during refining to produce a product that meets the required specifications. Distinct chemical differences were observed between the various fuels that were tested. This was elucidated by the 2-D gas chromatography (2-D GC) technique that was used. Conventional one dimensional gas chromatography, utilising only one column, cannot accurately separate all the constituents in diesel fuel since any single diesel may contain hundreds of thousands of individual hydrocarbon constituents (Mau *et al.* (2009); Adam *et al.*, 2010). This study illustrated the power of using 2-D GC in analysing diesel using this technique. The 2-D GC technique not only

produced a higher degree of separation, it also showed enhanced sensitivity to conventional 1-D GC techniques (Lee et al., 2001; Dallüge et al., 2003).

Most current research into the 2-D GC technique is focussed on further enhancing the capability and use of 2-D GC to identify and quantify organics in complex mixtures, it must however be noted though that even with this technique, there are still some shortcomings when elucidating middle distillate hydrocarbons from which diesel is produced. The 2-D GC technique cannot accurately discriminate between different paraffins, iso-paraffins, olefins, naphthenes and aromatics (PIONA) of middle distillates, which was evident in the results from this research. The different PIONA classes were well identified and quantified, however the more detailed separation was not seen, probably because the number of separation dimensions needed to separate hydrocarbons in middle distillates exceeds the number of separation dimensions of 2-D GC (Adam *et al.*, 2010).

The importance of 2-D GC from an environmental perspective is its use in the identification and quantification of hydrocarbons in remediation after pollution incidents. The importance of effective management of spills in the petroleum industry was well illustrated in the 2010 incident in the Gulf of Mexico where billions of litres of crude oil were spilled into the sea after a drilling operation went wrong. The impact of such and other petroleum related incidents can only be determined accurately when detailed and specific chemical information is available to determine the extent of the pollution and to assess the toxicity of the media in which the pollution occurred.

When pollution incidents occur it is not always practical to perform conventional toxicity tests since it may take several days to obtain results. Testing could also be limited to certain “standard” sentinel species that could sometimes not be relevant to the conditions of the specific incident; and it is not always practical and/or possible to obtain representative samples of the contaminated site. The value of predictive ecotoxicity potential was developed over the past few decades and although it can potentially save on response time to estimate the impact of a pollution incident, it is highly dependent on the quality and accuracy of the chemical analyses. Mau *et al.* (2009) utilised comprehensive 2-D GC (HPLC-GCXGC/FID) of oil contaminated soils and water, together with conventional ecotoxicological test in order to correlate between the ecotoxicity tests and the predicted ecotoxicity potential calculated from the 2-D GC analysis. The research indicated that more detailed HPLC-GCXGC/FID of the hydrocarbon constituents in the soil

and leachate, improved the ecotoxicity estimation of the soils phase and leachate water, enabling more accurate ecotoxicological risk models for petroleum hydrocarbon contamination in soils, sediment and water systems.

The use of 2-D GC techniques for petroleum hydrocarbon identification and quantification in engineered bioremediation processes (Penet *et al.*, 2006), biodegradation of hydrocarbons in inter-tidal zones (Slater *et al.*, 2006), has shown that by more accurate and detailed results, the fundamental understanding of the processes involved in hydrocarbon remediation are enhanced. This provides a better understanding of the persistence and risk of organics to biota in contaminated environments. From a risk management perspective it could provide tools that can be used to develop response plans for a variety of incident scenarios based on the category of use and transport. As well as determine the level of intervention needed once an incident has occurred, shortening response time and reducing the cost of initial clean-up and providing information on the impact and longer term remediation activities needed to minimise harm to the environment.

Adam *et al.* (2010) evaluated several novel approaches to overcome the major shortcomings of current 2-D GC techniques used to identify and quantify middle distillate hydrocarbon streams by which three-dimensional GC is proposed for PIONA analysis. These techniques will in future transform the capabilities and value of chemical analyses for organic constituents to be used in optimal toxicity evaluation and risk assessment of petroleum hydrocarbons in soils and water.

4.2. Biodegradation

4.2.1. Theoretical Oxygen Demand & Biodegradation Calculations

Battersby (2000) used the empirical formula of C_nH_{n+2} to determine the theoretical oxygen demand (ThOD) for the complete biodegradation of diesel samples. The present research calculated the ThOD using the data generated from the 2-D GC analysis and is presented in Table 3 of the Results chapter which showed that the ThOD decreases with a decrease in the number of hydrogen molecules in the hydrocarbon molecules contained in the diesel. The more paraffinic the diesel the higher the ThOD, which means that more oxygen (mass basis) is required to completely oxidise a molecule (mass basis) in the diesel to carbon dioxide (CO_2) and water. The ThOD of the biodiesel containing fatty acid methyl esters (FAME) had the lowest oxygen demand because the molecules contain oxygen in their structures and are partially oxidised.

The two crude oil derived diesels had ThOD's that were virtually identical, which is indicative of their composition. Both crude derived diesels contained high levels of aromatic compounds, mostly mononuclear aromatics, constituents required to comply with the diesel specification. The refining conditions and blending of diesels in a typical crude oil refinery would produce diesels of similar, but not identical, composition since the characteristics of the crude oil play a very important role in the final composition of the fuel products. The diesel quality is mainly driven by the SANS 342:2005 (2005) specification and hence the final products from crude refineries displayed ThOD's reflecting their composition.

The Sasol high temperature Fischer-Tropsch (HT-FT) blending materials, consisting largely of Distillate Hydrotreater (DHT) diesel, Distillate Selective Cracker (DSC) diesel, Creosote diesel and CatPoly diesel, together with other undisclosed materials to increase the density, showed varied ThOD values. The lighter DHT diesel had a ThOD identical to GTL diesel, because it also contained mostly paraffinic molecules. The heavier DSC diesel which is produced in a different process and contain aromatic constituents as well as paraffinic hydrocarbons had a ThOD close to that of the crude oil derived diesels. The Creosote diesel and CatPoly diesel, which are produced via refining of pyrolysis products from the gasification process contains high levels of branched paraffins and paraffins and low levels of n-paraffins. CatPoly diesel especially contains 99% branched paraffins and Creosote contains high levels of aromatic constituents. The production of these blending

materials is unique to the coal-to-liquids (CTL) process used in Secunda to produce diesel and other liquid fuels for the South African markets.

The significance of calculating the ThOD more accurately by using the 2-D GCMS results supported the biodegradability measurements using the manometric respirometer tests. Although Battersby (2000) used the generic formula for calculating the ThOD, the present research has shown that GTL diesel can have a ThOD value of between 3% and 3.5% greater than the ThOD of crude derived diesels. When testing diesel blending materials that is unique to Sasol's CTL processes, it is even more valuable to use the 2-D GCMS results to calculate the ThOD as opposed to using the generic empirical formula for paraffins because the molecular composition of the blending materials are not well known because of the limitations of previously used analytical techniques.

In the present research, which focussed on testing experimental diesel blends using various blending materials and biodiesel, the difference in ThOD of biodiesel and petroleum diesel compositions were illustrated. The importance of calculating the ThOD for the respective blending materials was demonstrated when measuring the biodegradation rates. The accurate measurement of biodegradation rate depends on the composition and the ThOD plays an important role. It is recommended not to use the generic calculation, but rather the calculation based on the molecular composition.

4.2.2. Biodegradability

The biodegradation results showed that the various diesels and diesel blends differ from one another in terms of biodegradation rates. The biodiesel had the highest biodegradation rate, followed by the GTL-derived diesel and CTL-derived DHT diesel. The CTL-derived DSC- and CTL- derived blending materials (Creosote and CatPoly) performed the worst and exhibited the lowest biodegradability rates in the biodegradability tests. The molecular composition of the various diesels could be the reason for the biodegradability performance of the diesels. According to Pitter and Chudoba (1990) biodegradation rates of hydrocarbons decreases in the following order: alkanes > branched alkanes > cyclo-alkanes > aromatic hydrocarbons. The biodegradability rates measured for the various diesels and diesel blends followed the trend as suggested by Pitter and Chudoba (1990) where the biodegradability rate decreased with increasing concentrations of branched alkanes, cyclo-alkanes and aromatic molecules. Table 20 illustrates the composition of each diesel and diesel blending material in terms of its hydrocarbon groups. GTL diesel and DHT diesel blending material both have a paraffinic composition, with the only difference being the ratio of n-paraffin / branched paraffin / cyclic paraffin ratio. Where GTL diesel is essentially a 42 / 58 / 0 ratio, the DHT is a 23 / 76 / 10 ratio. Jørgensen et al. (1998) suggested that aliphatic compounds are more biodegradable than aromatic compounds. The results of the present study confirmed this general biodegradation trends for hydrocarbons.

Table 20: The hydrocarbon groups of the diesel and diesel blending material that were tested for biodegradability.

	Paraffin			Aromatic [Bi- + Polynuclear]
	n-Paraffin	Branched Paraffin	Cyclic paraffin [Monocyclic]	
GTL Diesel	42	58	0	0
Natref Diesel	13	35	25 [18]	27 [5]
EN590	26	24	27 [18]	23 [4]
CTL DHT	23	67	10	1
CTL DSC	26	25	23 [12]	26 [2]
Creosote BM	12	2	57 [14]	29
CatPoly BM	1	91	7	1

Biodiesel had the highest biodegradability rate when compared to the petroleum derived

diesels. Hydrocarbons yield more energy, in the form of Adenosine-5'-triphosphate (ATP), than most organic compounds and vegetable oil is a main source of energy for aerobic cells (Metzler, 1977). Biodiesel molecules (Fatty Acid Methyl Esters) are directly derived from vegetable oils like the soybean oil from which the biodiesel used in the present study was derived. This process of assimilating the hydrocarbons takes part in the mitochondria of eukaryotic cells where fatty acids are drawn into the mitochondria under enzymatic catalyzed reactions. The initial “attack” of the fatty acids occurs at the oxidised (or ester) end of the molecule, after which β -oxidation is initiated to convert fatty acid chains into large amounts of ATP. Prince *et al.* (2008), Dermirbas (2007), Zhang *et al.* (1998), Mudge and Pereira (1999) and Schleicher *et al.* (2009) suggested that biodiesel, in mixtures or individually, biodegrades more rapidly than petroleum diesel. The current research confirmed that biodiesel biodegraded faster than the petroleum derived diesels. The mechanism of assimilating alkanes, cyclo-alkanes and aromatic molecules into the metabolism of the cells could provide some insight into the reason for the retarded biodegradability of petroleum derived diesels.

The proposed biochemical mechanism for paraffin digestion is made possible by the use of increased enzyme activity to facilitate fatty acid assimilation (Metzler, 1977). The saturated hydrocarbon molecule does not possess the oxygenated end of a fatty acid ester and hence the first step in paraffin oxidation is the hydroxylation of the molecule using molecular oxygen to produce a corresponding alcohol (e. g. n-octane will be hydroxylated to octanol). This is achieved via group-specific oxygenases that catalyses the introduction of molecular oxygen into the hydrocarbon molecules (Wilkinson *et al.*, 2002). Further oxidation accompanied by enzymatic action activates the molecule to enter the common catabolic pathways like for β -oxidation as in fatty acid oxidation to yield ATP (Metzler, 1977). Figure 32 illustrate the catabolism of an alkane like tridecane.

Branched hydrocarbons as well as fatty acids can block the β -oxidation mechanism when the branch is on an uneven carbon number position (e. g. 3, 5, 7 etc.), but will continue uninterrupted as long as the branch is on an even carbon number position (e. g. 2, 4, 6, etc.). When a blockage occurs, hydroxylation of the terminal carbon of that molecule takes place, which has the effect of retarding biodegradation. In certain cases where detergents were used in the past, before this mechanism was well understood and where the branches on the hydrocarbons were randomly distributed, such blockages were common. Cases have been reported where β -oxidation was blocked thereby preventing rapid

biodegradation and causing foaming at wastewater treatment works (Metzler, 1977). There are fewer microbial species that are able to metabolise branched hydrocarbons and it is believed that the branched molecules impede uptake into the cells and mitochondria. Although the microbes may have the ability to metabolise the branched hydrocarbons, they may still experience difficulty in producing the enzymes associated with β -oxidation (Wilkinson *et al.*, 2002).

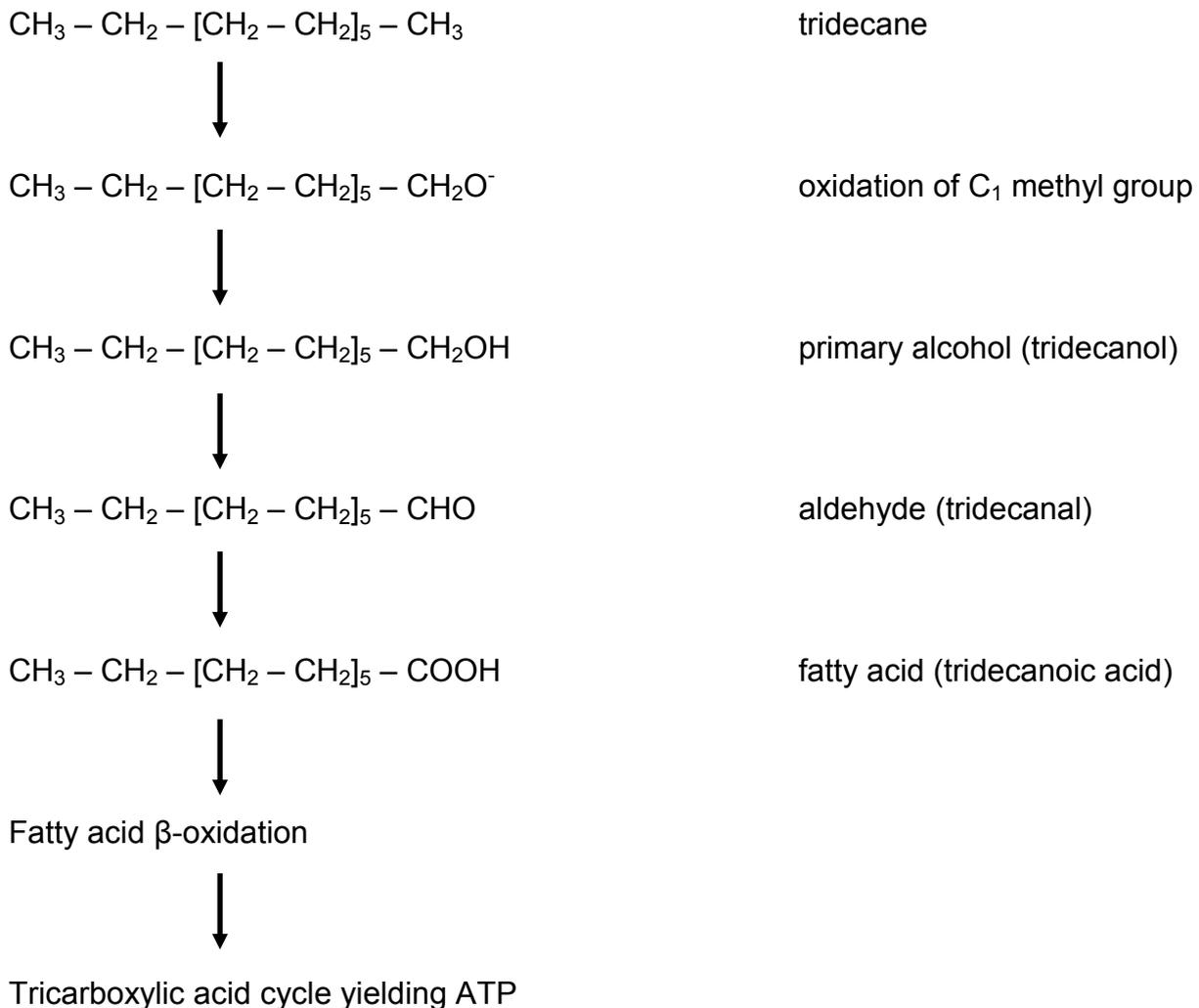


Figure 32: Metabolism of tridecane. Adapted from Wilkinson *et al.* (2002).

Cyclic paraffins undergo similar metabolic degradation processes, only it requires more complex enzymatic activity than alkanes. The cyclo-alkane is initially “attacked” by hydroxylase enzymes that leads to direct insertion of two oxygen molecules, followed by cleavage of the ring structure to produce a dicarboxylic acid (Prichard *et al.*, 1995). The more complex enzymatic activity retards biodegradation. The diesels containing greater than 20% cycloparaffins showed a lower biodegradation rate than those diesels with little

or no cycloparaffins.

The CatPoly diesel blending material containing more than 90% branched paraffins and less than 1% n-paraffins showed the lowest biodegradation rate (34.7%) after the 28 days exposure period of the test. The origin of the CatPoly diesel on a CTL facility is via the cold separation process that produces condensate material which is worked up into CatPoly diesel blending material at the CatPoly and Catpoly hydrotreater Units. The branching of this diesel blending material could have had the effect of retarding biochemical pathways with the resulting effect of rendering the material persistent in the aquatic environment.

Zhang *et al.* (1998) emphasised the effect of the availability of the appropriate enzymes for the biochemical processes to occur rapidly. Biodiesel FAME molecules require less enzymatic activation because of the direct mechanism at the oxidised end of the ester, whereas in saturated hydrocarbons, hydroxylation of the terminal carbon is needed before initialisation of β -oxidation can occur. In addition, most fatty acids are even hydrocarbon chains which make them inherently biologically active molecules (Zhang *et al.*, 1998).

The two crude-derived diesels, Natref diesel and EN590 reference diesel, as well as the DSC and Creosote diesel blending materials contained between 23% and 29% predominantly aromatic constituents. Natref diesel and EN590 reference diesel and DSC diesel blending material contained 5%, 4% and 2% bi- and polynuclear aromatics respectively (Table 20). The three diesels showed similar biodegradation rates of 43.92%, 47.61% and 53.57% for the crude derived Natref and EN590 diesel and the DSC blending material respectively after the 28 day exposure period. None of the three diesels reached the 60% biodegradation before the 28 day cut-off of the test and hence could not be classified as “ready biodegradable” according to the test method. These diesels could be considered as “inherently biodegradable” since they did show significant biodegradation and continued biodegradation is expected after 28 days. The effect of the more complex molecules like branched and cyclic alkanes as well as mono-, bi- and polynuclear aromatic constituents could have retarded biodegradation rates of these diesels.

According to Wilkinson *et al.* (2002) aromatic molecules are broken down by similar mechanisms as cycloparaffins whereby intermediate molecules are produced via enzymatic catalysed reactions by central metabolic routes, dioxygenation and ring scission.

The required production of enzymes and transformation of intermediate molecules to activate catabolism increases the time it takes to metabolise hydrocarbons as well as the selectivity of microbes to initiate assimilation of the more complex and stable hydrocarbon molecules as food source. Other important factors influencing biodegradation of aromatic compounds in aquatic environments are physical-chemical such as pH, temperature, the availability of oxygen for respiration and the availability of macro- and micro nutrients.

Despite all the difficulties of biological degradation of hydrocarbon, microbes have developed the ability to assimilate it as a food source. In environments where hydrocarbons are abundant, such as hydrocarbon polluted soils or point source discharge of hydrocarbons into water courses, the abundance of hydrocarbon degrading microbes are higher than in areas where hydrocarbon pollution is absent. Atlas and Bartha (1992) used the term “hydrocarbonoclastic” to describe hydrocarbon utilising micro-organisms. It is believed that small amounts of hydrocarbonoclastic microbes are present in all environments and when hydrocarbons become available, either through anthropogenic activities or natural occurring events, these microbes will be selected and increase in abundance to utilise the hydrocarbons as food source should environmental conditions be favourable. This ability of micro-organisms to adapt to hydrocarbon contamination has been widely used to combat hydrocarbon pollution by biological remediation of contaminated soil and water using acclimatised microbes (Prince et al., 2008). The result is mineralisation of often toxic and seemingly persistent hydrocarbons to carbon dioxide and water.

Addition of biodiesel to crude oil derived diesel increased the biodegradation rate. The improvement in biodegradation rates was observed at 12 days, which is normally the cut-off time for the 10-day window period according to the test methodology. None of the biodiesel/EN590 reference diesel achieved the 10-day window pass criteria and could not be classified as “readily biodegradable”, although the 30% biodiesel blend did achieve greater than 60% biodegradation after 28 days. Pasqualino *et al.* (2006) found that the addition of biodiesel to diesel and gasoline improved the biodegradability of the mixtures and concluded that the addition of biodiesel could be successfully used as a solution in the bioremediation of fossil fuel contaminated sites. The results from the present study indicated that even small amounts of biodiesel addition to crude oil derived diesel increased the biodegradability rate of the blended material when compared to the crude derived diesel on its own. A reason for this phenomenon is that biodiesel can behave like

a non-volatile organic solvent when blended with hydrocarbon mixtures. Mudge and Pereira (1999) found that biodiesel is able to dissolve the longer chain aliphatic- and polyaromatic hydrocarbons and make them more mobile in the environment. Furthermore, Miller and Mudge (1997) and Pereira and Mudge (2004) demonstrated the usefulness of biodiesel in the removal of crude oil from intertidal sediments and attributed it to the solvent properties of the biodiesel. The solvent behaviour of the biodiesel blends with crude derived diesels could make the hydrophobic aliphatic and aromatic molecules more bioavailable to the microorganisms in the biodegradation test flasks, enhancing the biodegradability of the blend.

The CTL DSC diesel had a similar composition to that of both the Natref Final diesel and EN590, both crude-derived diesels, yet had a higher biodegradation rate than both. The CTL DSC Heavy diesel exhibited the highest variability of the complete diesels. An explanation for this phenomenon could be found in the composition. The CTL DSC Heavy diesel contained less monocyclic, bicyclic, bi-aromatic and polyaromatic constituents. The fact that more were present in the two crude-derived diesels could have retarded the biodegradation when compared to that of the CTL DSC heavy diesel.

The biodegradation of the two crude-derived diesels did not correspond and the composition of the diesels could be the reason for this as well. The Natref Final diesel contained almost half the amount of n-paraffins when compared to the EN590 reference diesel. In addition, the EN590 diesel contained approximately 10% less branched paraffins than the Natref Final diesel. The increased concentration of branched paraffins, in all probability, was responsible for the difference in biodegradation rate between the two crude-derived diesels.

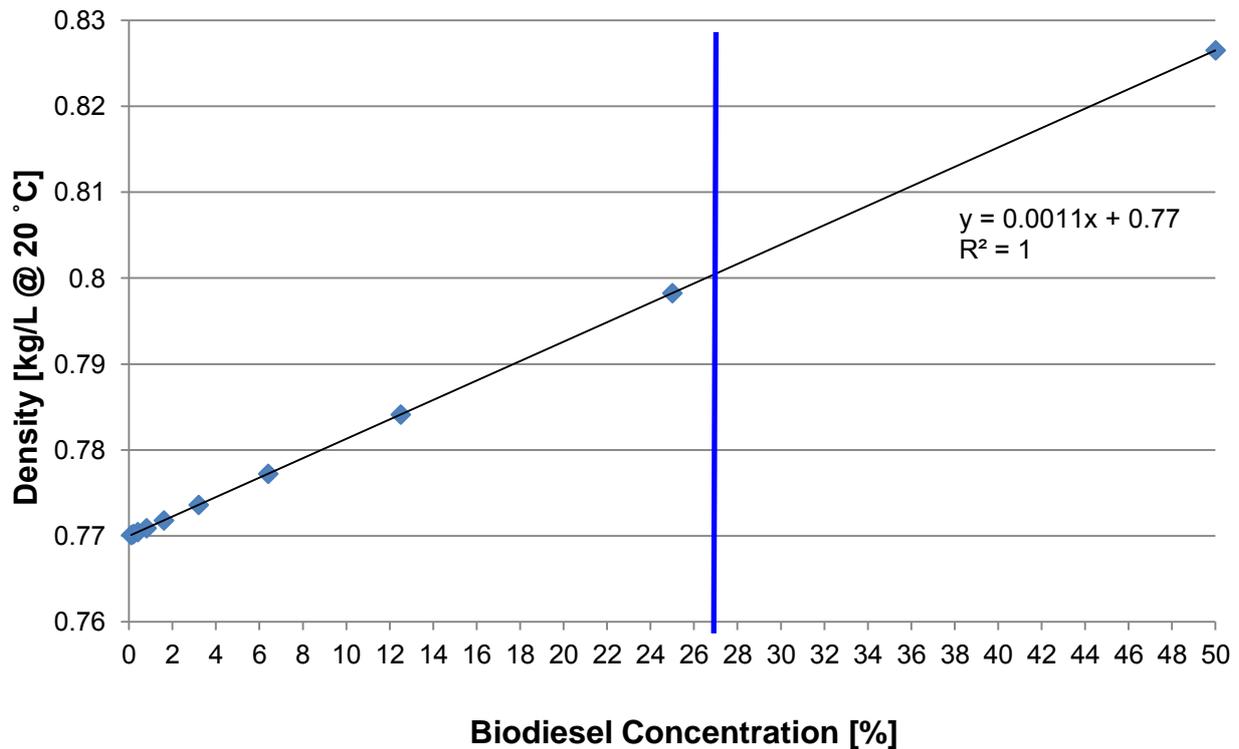
The testing of biodiesel/GTL diesel blends was explored to observe the effect of biodiesel addition on the GTL fuel. The biodegradation rates increased with an increase in biodiesel addition. Statistically biodiesel addition of 0.8% reached greater than 60% biodegradation within the 10-day window as well as greater than 70% biodegradation at the end of the 28-day test period. Regression analysis using average biodegradation rates at 12 days and 28 days of the tests, indicated that as little as 0.28% soybean derived biodiesel is required to achieve the 10-day window requirement of the test method and that 2.69% soybean derived biodiesel is required to achieve 65% biodegradation within the 10-day window to account for variability in the biodegradation test. The trend of increased biodegradability

with increased biodiesel addition to GTL diesel is remarkable, but not unexpected, since the biodegradability of the biodiesel on its own had a biodegradation rate approximately 20% higher than that of the GTL diesel on its own. The results presented in the present study can expand the biodegradation hierarchy of Pitter and Chudoba (1990) to include the fatty acid methyl esters (FAME) where biodegradation rates of hydrocarbons decreases in the following order: FAME > alkanes > branched alkanes > cyclo-alkanes > aromatic hydrocarbons. The biodiesel/GTL diesel blends with biodiesel concentrations exceeding 0.4% achieved average biodegradation rates greater than 60%; however the individual replicates showed high levels of variability, which is not uncommon for petroleum substances biodegradation tests as reported by Clark et al. (2003) and Battersby et al. (1992).

The effect of biodiesel addition appears to have promoted or enhanced the biodegradation of petroleum derived and synthetically produced diesel, which confirmed the research of Zhang *et al.* (1998) and Pasqualino *et al.* (2006). Research of Taylor and Jones (2001), Wilkinson et al. (2002), Pereira and Mudge (2004), Horel and Schiewer (2011) suggested that the addition of biodiesel to contaminated soils and sands can enhance the biodegradation of the crude diesel contamination. Cyplik *et al.* (2011) put forward that the degraders in the microbial consortium responsible for the degradation of biodiesel remain generally the same as those for crude-derived diesel. From a biochemical perspective, the biodegradation results from the present study confirmed that biodiesel-mediated biodegradation of petroleum hydrocarbons can be achieved. Biodiesel enhances biodegradation, not only by degrading the biodiesel first, but by simultaneously degrading the hydrocarbons as well and at the same time adapting the process of metabolising hydrocarbons. So when the biodiesel fraction is consumed, the remaining hydrocarbons can be consumed more rapidly.

The results from the present research indicated that using biodiesel could improve the GTL diesel biodegradability to the extent that it can be classified as “ready biodegradable”. One of the important aspects of GTL diesel is its density that is below the diesel specification (SANS 342, 2005). It is known that biodiesel has higher density than petroleum diesel with soybean derived biodiesel having a density of 0.883kg/L at 20°C as opposed to the 0.777kg/L at 20°C of GTL diesel. Regression analysis of biodiesel/GTL diesel blends indicated that to achieve the density specification range of SANS 342 (2005) of 0.8000kg/L at 20°C, a minimum of 27.3% soybean derived biodiesel addition would be

required (Fig. 33).



◆ Theoretical density of Blends @ 20 °C — Linear (Theoretical density of Blends @ 20 °C)

Figure 33: Regression analysis illustrates that as much as 27.3% of soybean derived biodiesel addition to GTL diesel is required for the blend to achieve the minimum density requirement for the SANS 342 (2005) specification.

The addition of biodiesel to petroleum diesel as an additive to increase lubricity of low sulphur diesels has been shown (Van Gerpen *et al.* 1999) since low sulphur diesel exhibit reduced lubricity compared to the high sulphur diesels of the past (Anastopoulos *et al.* 2001). Although biodiesel can negatively influence the viscosity when blended with petroleum diesel, the low concentrations needed to act as a lubricity enhancer (less than 5%) would not reduce the viscosity with the added advantage of negative impact on the exhaust emissions' harmful gasses (Goodrum and Geller, 2005). At the levels of biodiesel required to enhance the lubricity of GTL, the biodegradation of the diesel will be enhanced to the level at which it would be classified "readily biodegradable" passing the 10-day window. The addition of biodiesel will also increase the diesel density to a small extent, but not enough to comply with the specifications for biodiesel in South Africa.

The addition of biodiesel to GTL or other petroleum derived diesels does not seem viable at this stage since the production of biodiesel is more expensive than petro-diesel and the availability of land to produce the oil from oilseeds is not sufficient in SA (Nolte, 2007). According to Nolte (2007) who conducted an economic evaluation of biodiesel production for a SA scenario and suggested that approximately 6.5 million tons of soybeans will have to be produced in addition to the existing production to meet a 10% blending addition of 1 199 million litres to petro-diesel. This would require an additional agricultural area of approximately 4 million hectares for cultivating the beans and will ultimately also require additional production facilities. If only 5% biodiesel were to be produced, considering its use in increasing the petro-diesel lubricity, one would still require approximately 2 million hectares of soybean cultivation agricultural land.

Given the intrinsic benefits that biodiesel may have in terms of its lubricity and biodegradation rate, addition to GTL-derived diesel could be more environmentally acceptable. By putting well-defined policies and incentives in place, a robust biodiesel industry could be created that will enable SA to contribute to the mitigation of the threat of climate change, to become less dependent on foreign oil and to develop rural agriculture.

The first hypothesis is supported in its entirety because of the following reasons:

- The present study has shown that the composition of the diesel, diesel blending material, biodiesel and petro/biodiesel blends have an influence on the biodegradation rates.
- The biodegradation rates of the diesel fuels could be ranked accordingly where biodegradation rate decreases in the following order based on its composition: FAME > alkanes > branched alkanes > cyclo-alkanes > aromatic hydrocarbons.

4.3. Water Accommodated Fraction

The hydrocarbons contained in petroleum diesels can vary in terms of molecular weight, carbon number and the degree of saturation which can all have an effect on the water solubility of the individual hydrocarbons in the fuel mixture (CONCAWE, 2001). When a petroleum fuel, such as diesel, is added to water, at a certain concentration, the solubility limit for the constituent with the lowest solubility will be reached and exceeded. The remaining constituents will partition between the water and the undissolved hydrocarbon phase (Peterson, 1994; Cline *et al.*, 1991; Shiu *et al.* 1990).

This partitioning behaviour presents a challenge when trying to measure or estimate the actual hydrocarbon concentration in WAF's prepared from hydrocarbon fuels such as diesel. It is difficult enough to measure the diesel constituents in a diesel sample as was discussed in a previous section, let alone measure the actual fraction of the hundreds of hydrocarbons in the water fraction. Since the water soluble fractions of the hydrocarbons contained in the WAFs were not measured, the effect of the WAFs were theoretically calculated using techniques and concepts established during the early development of mathematical models over twenty years ago (Peterson, 1994; Cline *et al.*, 1991; Shiu *et al.* 1990) and refined in recent times (Fisk, 2005).

The toxicity to biota is linked to the hydrocarbon concentration dissolved in the water and is therefore important to know the constituents and the amounts at which they are present in the WAFs in order to interpret data and the impact of hydrocarbon mixtures in the environment (CONCAWE, 2001). Because a single substance is not responsible for the toxicity, it is known as non-specific narcotic mode of action (Abernethy *et al.*, 1988), which will be discussed in further detail in the Ecotoxicity Tests section below.

4.4. Ecotoxicity Tests

4.4.1. Aquatic Toxicity Tests

The general lack of severe toxic effect of the WAF's of the different diesels and blends for all the test species was remarkable. No acute toxicity, in other words 0% mortality, was observed in any of the fish tests at WAF loading concentrations of up to 1 000mg/L. The *D. magna*, algae and bacterial tests showed low acute toxicity where acute toxic effects were measured at equal to or less than 20%, which was similar to that recorded for undiluted Biodiesel. Poorly soluble complex organic mixtures such as diesel demonstrate selective partitioning behaviour between the water media and the hydrocarbon media. The result is a water soluble fraction of the diesel containing many different substances at very different concentrations, each potentially having an effect on the biota. The mechanism for acute toxicity that diesel and other hydrocarbon containing mixtures exhibit has been described as non-specific narcosis (Abernethy *et al.*, 1988; Van Wezel and Opperhuizen, 1995; Escher and Hermens, 2002; Öberg, 2004). The sensitivity of aquatic organisms to hydrocarbon mixtures like crude oil, diesel and gasoline has been largely attributed to the aromatic constituents in the mixtures (Anderson, 1977; Connell and Miller, 1981; Spies, 1987; French, 1991; Neff and Stubblefield, 1995; Pelletier *et al.*, 1997). Since the narcosis mode of action is additive, all the soluble constituents contribute to the toxicity of the mixture (Peterson, 1994; Barron *et al.*, 1997).

The aquatic ecotoxicity tests conducted during the present research were static non-renewal media tests. Barron *et al.* (1999) established in their research that up to 50% of the volatile hydrocarbons were lost between renewals of the media. The volatilisation of constituents from the water media could be the reason for the general lack of acute toxicity, i.e. the physical loss of hydrocarbons from the testing system. A contributing factor to the lack of acute toxicity could have been the loss of the more volatile fraction of the diesels. Peterson (1994) suggested that the toxicity of hydrocarbons is inversely related to its solubility where alkanes in solution are more toxic than the equivalent amount of benzenes in the solution. Although the conditions in reality (not laboratory) are changeable and several environmental factors could influence the fate of the diesel, it is important that the concentration of the test media is kept as constant as possible in experimental systems. This can be achieved through static renewal tests. The diesels tested in the present study all consisted of more alkanes and less aromatic constituents. It is plausible that the amount of aromatics relative to the alkanes could have been less as a

result of the partitioning behaviour of the hydrocarbons between the water, hydrocarbon mixture (diesel) and the air (volatilisation). The possibility exists that the organisms were not exposed to the same concentration of hydrocarbons throughout the experiments because of the loss of organics through volatilisation.

The petroleum diesels WAF's were the least toxic to the sentinel species tested. Those of the biodiesel and biodiesel blends showed the highest toxicity to the crustaceans, algae and bacteria tested. The biodiesel WAF's had a milky appearance. This could have been some of the FAME molecules emulsifying in the water. The lower volatilisation potential of the biodiesel also would have kept the molecules in water suspension for longer. Khan et al. (2007) employed static non-renewal to determine the toxicity of biodiesel (derived from used vegetable oil) and biodiesel blends on *D. magna* and *Oncorhynchus mykiss* (rainbow trout) and the biodiesel to be less toxic than the petroleum diesel and biodiesel blends. The present study found that the biodiesel containing diesels were more toxic, although the ecotoxicity was low and for *D. magna* the EC₂₀ could be calculated, but not the LC₅₀ because more than 50% of the test organisms did not show an immobilising effect. Furthermore, a toxicity ranking for the present study could be expressed with decreasing toxicity as: FAME > alkanes > branched alkanes > cyclo-alkanes > aromatic hydrocarbons. Whale et al. (2001) in studies of hydrocarbon products on earthworms and plants ranked the hydrocarbon toxicity expressed with decreasing toxicity as aromatics > cyclo-alkanes > branched alkanes > linear alkanes. This is the opposite of the ranking of the present research. Research by Peterson (1994), Barron et al. (1999) and Sandermann (2008) suggested that paraffinic molecules are more toxic than aromatic molecules. Donkin et al. (1998) found that notwithstanding the short environmental half-lives of aliphatic molecules with less than 10 carbons, tissue data in marine mussels showed that these molecules were taken up by the molluscs and accumulated and hence contributed to acute and chronic toxicity.

It is likely that many studies in the past focussed on aromatics because of their persistence in previously oil contaminated pollution that has weathered over time. The alkanes may have been present in lesser amounts, but still contributed significantly to the toxicity. The volatility of paraffinic molecules resulted in it being lost either from experimental systems or analytical samples. In an effort to understand the low acute ecotoxicity results obtained in the present study, one should not underestimate the potential of hydrocarbon containing

mixtures to have a chronic toxicity potential which was not readily observed in the performed tests.

The second hypothesis supported the following:

- The petroleum diesels and biodiesel showed no acute toxic effect to fish, while a slightly higher ecotoxicity was observed for crustaceans, algae and bacteria irrespective of the composition thereof.

The third hypothesis supported the following:

- The biodegradation rate after biodiesel addition to the petroleum diesels increased the biodegradability of the blends.

The third hypothesis was rejected where:

- The addition of biodiesel to petroleum diesel did not have a remarkable effect on the ecotoxicity to the sentinel species that were used and although a response was observed in most blends with biodiesel, the response was insufficient to calculate a median effect loading (EL₅₀).

4.5. Quantitative structure-activity relationship

4.5.1. Ecotoxicity Estimation

The results from the Fisk Ecotoxicity Estimation Model (FEEM) model showed that fish and daphnids were less negatively affected while the algae were estimated to be most affected at both 100mg/L loading and 1 000mg/L loading. In all the estimations of all the diesels, none of the fish or crustacean acute toxicity unit (TUa) results reached 1, which indicated that the median effect limit (EL₅₀) would not be achieved. These predictions corresponded with the ecotoxicity experimental results for the WAF's of the petroleum diesels. The highest ecotoxicity was observed in the experimental tests performed on the biodiesel and blends that contained biodiesel. The experimental results and the predictions did not correspond since the FEEM predicted the biodiesel to have the least toxicity based on the TUa for all three species modelled. This prediction influenced all the calculations where biodiesel was blended with the petro-diesel.

The reason for this inconsistency between experimental and estimation data could be found in the way the model calculates the TUa which is essentially based on the hydrocarbon-water partition K_{HCW} coefficient derived from the water-octanol partition coefficient K_{OW} . The K_{OW} values for the biodiesel constituents were all greater than log 7, which indicates that the biodiesel constituents are hydrophobic and have low water solubility. The low total estimated exposure of the biodiesel was the results of the calculations. The fact that the biodiesel WAFs had a milky appearance could lead one to believe that the biodiesel either has higher water solubility than estimated or can readily form emulsions in water. Girling *et al.* (1992) suggested that compounds that readily emulsify in water, without the input of energy, can be considered soluble in water. The milky appearance in the biodiesel and biodiesel blends could have been the emulsion that formed and the organic hydrophobic constituents behaved like water-soluble molecules. The emulsification could not be predicted in the calculations of the biological effect of biodiesel and hence influenced all calculations of the products where biodiesel was added to the petro-diesel. This was evident in the TUa values of the biodiesel blended predicted dose responses. The major factors affecting toxicity of hydrocarbons in organisms are the size of the molecules and the molar volume reaching the target site in the body (Abernethy *et al.* 1988). The hydrophobicity of a compound is related to its K_{OW} , and the "cut-off" for the particular compound having a significant effect on an aquatic organism is considered to be a K_{OW} of log 7 based on the size of the molecule and its low water-solubility. For all

the calculations of the present study using the FEEM, a K_{OW} smaller than log 8 was included.

In studies by Peterson (1994), Barron *et al.* (1999) and Sandermann (2008), it was pointed out that paraffinic compounds could contribute more to the toxicity of the mixture, for example the research by Whale *et al.* (2001) who suggested that the aromatics were more toxic than aliphatics. The FEEM model was used to group the individual molecules into units of similar molecular structures and found that in all the diesels, the paraffinic compounds contributed more to the TUa than the aromatic molecules. The GTL, DHT Light and CatPoly diesels consisted almost entirely of paraffins and it was expected that any toxicity would be as a result of the paraffinic molecules. The diesels containing between 22% and 29% (by mass) aromatic molecules were considered ideal candidates to test the potential effect of the hydrocarbon groups. The predictions indicated that the paraffins did contribute more to the TUa than the aromatics. The FEEM could also show which group was estimated to have made the biggest contribution to the TUa for each diesel.

The results from the FEEM demonstrated that when working with complex mixtures, the QSAR estimations can be useful in providing detailed support on the potential effects of environmental contaminants like hydrocarbons. When dealing with complex mixtures, especially mixtures containing compounds with low water-solubility, it may not be feasible to test each individual compound and the use of QSARs becomes more feasible and can be used together with biological effect models that incorporate the “additivity” in their assumptions to predict the cumulative effect of the mixture, viz. FEEM (Rufli *et al.* 1998).

4.5.2. Biodegradation estimations

The biodegradation model estimated the biodegradation rates for the complete diesels accurately. The accuracy of the predicted Ultimate Biodegradation according to the Episuite software for the complete diesels was variable. Four out of the eight complete diesels predictions corresponded with the experimental results as opposed to two diesels each that were underestimated and underestimated by the biodegradation model. As with the ecotoxicity estimations the influence of the K_{OW} estimation for the biodiesel resulted in a lower Ultimate Biodegradation prediction and also indicated that none of the biodiesel constituents were regarded as being “readily biodegradable”, passing 10-day window

(RB). This factor influenced all the diesels that contained biodiesel and only four out of the 16 biodiesel/petrodiesel blends corresponded with the experimental biodegradation results. Incidentally those four diesels contained the lower biodiesel percentages in the blend and ranged from 0.1% and 0.2% in the biodiesel/GTL diesel blends to 5% and 10% in the biodiesel/EN590 blends.

The Episuite model could be considered inaccurate in estimating the biodiesel biodegradability and a correction factor could be used to reduce the error between the experimental and predicted results. The correction factor that was chosen was to assign the biodiesel fraction a 100% RB classification based on the fact that its 28-day biodegradation rate that was measured during the present study was equivalent to that of the sodium acetate, which was used as a reference substance indicating ready biodegradability. When this correction factor was applied, the biodegradation prediction for the blends containing biodiesel changed and resulted in only one biodiesel/petrodiesel blend (30% biodiesel, blended with 35% GTL diesel and 35% EN590 diesel) (Table 21) changing tiers up. It is remarkable that it was still underestimated, since the experimental blend passed the 10-day window and was classified RB while the predicted classification only moved up one tier to IB from IBN.

Table 21: Biodegradation estimation results, with biodiesel biodegradation corrected value, and experimental biodegradability rates of the diesel blends. The estimation results (Est. Result) indicate whether the predicted values Corresponded (C), Underestimated (U) or Overestimated (O) the experimental values. The highlight indicates the change in classification.

Diesel	BioWin 3 Estimate	Biodegradability result		Exp. Biodegradability [%]		Biodegradability result	Est. Result
		With CF	Without CF	12 Days	28 Days		
5% Biodiesel addition to EN590 diesel	3.15	IBN	IBN	38.43 ± 1.3	52.82 ± 1.9	IBN	C
10% Biodiesel addition to EN590 diesel	3.15	IBN	IBN	40.37 ± 2.3	55.88 ± 3.6	IBN	C
30% Biodiesel addition to EN590 diesel	3.15	IBN	IBN	48.43 ± 1.8	63.53 ± 3.2	IB	U
0.1%Biodiesel addition to GTL diesel	3.15	RBN	RBN	58.39 ± 1.4	69.43 ± 4.2	RBN	C
0.2% Biodiesel addition to GTL diesel	3.15	RBN	RBN	57.61 ± 4.8	69.08 ± 4.1	RBN	C
0.4% Biodiesel addition to GTL diesel	3.16	RBN	RBN	62.35 ± 6.3	76.20 ± 3.9	RB	U
0.8% Biodiesel addition to GTL diesel	3.17	RBN	RBN	60.60 ± 2.7	73.55 ± 7.7	RB	U
1.6% Biodiesel addition to GTL diesel	3.19	RBN	RBN	64.68 ± 3.6	79.48 ± 2.1	RB	U
3.2% Biodiesel addition to GTL diesel	3.22	RBN	RBN	66.20 ± 5.4	80.90 ± 3.6	RB	U
6.4% Biodiesel addition to GTL diesel	3.29	RBN	RBN	66.77 ± 3.0	80.64 ± 4.1	RB	U
12.5% Biodiesel addition to GTL diesel	2.21	RBN	RBN	68.00 ± 2.5	81.46 ± 4.0	RB	U
25% Biodiesel addition to GTL diesel	2.28	RBN	RBN	69.16 ± 2.1	77.42 ± 2.5	RB	U
50% Biodiesel addition to GTL diesel	2.53	RBN	RBN	74.18 ± 4.4	83.21 ± 3.2	RB	U

5% Biodiesel, 47.5% GTL diesel, EN590 diesel	2.69	IBN	IBN	49.40 ± 3.4	60.26 ± 2.8	IB	U
10% Biodiesel, 45% GTL diesel, EN590 diesel	2.73	IBN	IBN	52.97 ± 4.5	60.85 ± 3.9	IB	U
30% Biodiesel, 35% GTL diesel, EN590 diesel	2.88	IB	IBN	59.94 ± 2.2	69.49 ± 1.2	RB	U

The correction of the biodiesel estimate did not affect the classification to correspond to the experimental classification of the biodiesel/petrodiesel blends. The present research into the applicability of Episuite estimation Ultimate Biodegradation model is questioned and although the model and experimental classification for petrodiesels and diesel blending materials were not entirely inaccurate, the estimations were one tier over- or underestimated.

The CTL DSC Heavy diesel was classified as IB material experimentally and the present research indicated that the composition could have been responsible for this classification. The two blending materials, Creosote diesel and CatPoly diesel, were both overestimated and classified as IB and RBN respectively whereas the experimental classification was IBN for both. It could be argued that because of the incorrect estimations and resultant classification of the blending materials as well as biodiesel, which could also be regarded as a blending material, the Episuite software and biodegradation model used in the present study is best used in complete diesels. This seems to be the apparent indications when looking at the overall classification, however when looking at the following diesels' relative classifications in the order of decreasing biodegradability: Natref Final > CTL DSC Heavy > EN590, it was apparent that when compared to the experimental biodegradation rates, it was not estimated correctly. Relatively, Natref Final diesel was overestimated and both CTL DSC Heavy diesel and EN590 reference diesel were underestimated and the experimental biodegradation ranking in order of decreasing biodegradability can be expressed as: CTL DSC Heavy > EN590 > Natref Final.

While the relative estimations were inaccurate, the model is still applicable in terms of the general trend for the biodegradation. The biodegradation model used in the present study would be applicable to diesel blending research without biodiesel addition. The model would be used to estimate the overall biodegradability of new petrodiesel blends as screening tool before blending material production process changes are proposed and/or when a change in diesel specification is expected to change the hydrocarbon composition of the blending materials. This model is not yet optimised for biodiesel addition and hence is not advised to be used for the purpose of screening for biodegradation prediction where biodiesel is a blending material.

Ultimately the biodegradation prediction model should be refined and adapted to predict the biodegradation more accurately and some development is still required. It is useful to

note that environmental models are being optimised with increasingly sophisticated software and expanding experimental databank information which could make risk assessment of mixtures more rapid as well as less expensive. This together with current and future developments in analytical capability will further enhance the power of estimation tools. Walker (2003) believed that the use of QSARs for pollution prevention is driven primarily by economics and can be useful to chemical producers when developing products as well as governmental organisations that ultimately regulate those manufacturers.

The fourth hypothesis was supported because:

- The ecotoxicity estimations were accurate for the fish and crustaceans,

The fourth hypothesis was rejected where:

- The ecotoxicity estimations were inaccurate for the algae.
- The biodiesel toxicity was underestimated
- The total hydrocarbon concentrations were inaccurately estimated in the WAF's.
- The biodegradation of the estimations were inaccurate to estimate the overall and relative biodegradation rates when compared to the experimental results.

4.6. Of QSAR's and Aquatox: An Ecotoxicity and Biodegradability Evaluation

The present research also evaluated diesels, diesel blending materials and biodiesel/diesel blends by measuring the ecotoxicity and biodegradability of the materials experimentally as well as estimating the same parameters using a combination of QSAR estimation and modelling methods. What made this research very complex from both an experimental and modelling perspective were the fuel mixtures that contained from as little as three major constituents to others containing more than a hundred individual constituents. With current technology it is impossible to experimentally simulate or measure every parameter (physical, chemical or biological) of every process in nature, together with all the accompanying interactions and responses of all living organisms affected by a test substance.

This section of the present research compares the two approaches that were used. The present research and similar industrial research into the development of environmentally more acceptable products, does not necessarily aim to reduce the environmental impact of existing products, remediate the impact of existing pollution or prevent the impact of current products on the environment. Rather, it aims to develop new products that comply with the latest or future environmental requirements: products that are socially more acceptable in terms of their perceived impact and/or products that provide better product performance without additional environmental impact. New product development is important and competition between manufacturers can be fierce. Approximately 50 000 new substances are being registered on the CAS registry (CAS registry, 2011) every week indicating the pace at which new products are being evaluated for use.

The question is which one of experimental measurement or QSAR modelling is the best tool to evaluate products. During the present study approximately 96 hours were required to test a batch of four diesels experimentally for biodegradation rate and ecotoxicity. The estimation procedure with QSAR and FEEM modelling of the same batch of diesels took only 24 hours. The estimation route was approximately four times less time consuming than the experimental measurement route. That indicates that the estimation route is less costly than the experimental route. Estimation models are by default inaccurate and there is a level of uncertainty in the output that can range between 20% and 50% (Jørgensen *et al.* 1998). There is however also a level of uncertainty or more specific, a variability in experimentally produced data that could vary between 20% and 80% (ISO 11348-3, 1999),

depending on the test that is being used. Generally a variability of less than 10% is considered acceptable in the control for the OECD tests that were used during the present research.

Jørgensen *et al.* (1998) suggested that the aim of estimation models is to indicate how far from harmful or very harmful a substance is. In practice, for substances that have a greater potential to cause harm to ecosystems, a large safety factor can be applied to protect the biodiversity of the ecosystems. The concept of safety factor is not applicable during product development, but estimation models are rather used to screen many different blends, formulations and products in relatively short periods of time. The objective is to “shortlist” the long list of potential formulations by filtering the new products in three categories:

1. Continue to next tier of development which is normally experimental assessment
2. Stop development based on either poor product performance or worse environmental impact
3. Measure/estimate more accurately and then filter via 1 or 2

Once a list of potential products has been identified, manufacturing of the “new” product, normally at pilot scale, can begin and experimental measurement of product performance and environmental impact can commence. The use of the modelling tools is there to screen many different substances in a relatively short period of time and is the major advantage of the QSAR tools. This is demonstrated by the use of selected tools like Episuite that is used by the US EPA to review thousands of chemicals in an attempt to determine the possible toxicological effects to the biological environment and to human health, and the premanufacture notices (PMN) submitted to the US EPA for approval (Raymond *et al.*, 2001).

The use of QSAR's in environmental risk assessment has reduced the cost and time saved, however with many different QSAR's available as well as the fact any one QSAR cannot be applied to all substances, some guidelines on their use was inevitable, especially from a regulatory perspective. To ensure consistency of application of the Environmental Risk Assessment (ERA) process, in 1996 the EU produced a comprehensive technical guidance document (TGD) to support the Directive on New Substances and the Regulation on Existing Substances (EC, 1996). This document includes a substantial chapter providing guidance on the use of QSARs in the ERA

process in terms of where they should be used, how they should be used, and which ones should be used (Comber et al., 2003). The EU TGD recommended four specific uses of QSAR's in environmental risk assessment (Comber et al., 2003):

1. Data evaluation – acceptable QSARs may be used as a supporting tool to evaluate the adequacy of the available experimental data, for example, when the validity of the test data is not obvious. This may occur when incomplete data are available on the test and/or the test differs in some way from current methods, for example, OECD test guidelines.
2. Decision for Further Testing/Testing Strategies – in those cases where a predicted environmental concentration (PEC)/predicted no effect concentration (PNEC) ratio, established using test data, is greater than 1, there will be a requirement to determine whether additional testing is needed to allow a refinement of the PEC/PNEC ratio. To facilitate this decision, all available test data should be reconsidered along with estimates established using acceptable QSAR's. If PEC/PNEC ratios derived using the QSARs suggest that further testing is required, then generally a chronic test should be conducted on the species that showed the lowest estimated no-observable-effect concentration (NOEC).
3. Establishing Specific Parameters – acceptable QSAR's can be used for the estimation of specific (input) parameters used in the risk assessment, particularly in the exposure assessment when no measured data are available to enable derivation of the PEC.
4. Identifying Data Needs on Effects of Potential Concern – acceptable QSAR's can be used for preliminary assessment of endpoints that are not part of the base set of data and for which information is not available.

Wider acceptance of QSAR generated information and data would result in savings to both private (chemical manufacturers) and public (regulatory) sectors (Tong et al., 2005). From the present research, where it was shown that QSAR models can estimate biodegradability and ecotoxicity information with reasonable accuracy, it can be concluded that the use of both estimation models and experimental measurement have a place in new product development. Although the use of estimation models provides a relatively quick and cost-effective way to screen many products, the experimental measurements can be used to confirm or reject the model results. The comparison of the results of the biodiesel biodegradation model and the experimental results is a good example of where the difference between the two approaches can be found. The combination of using

estimation models in the early stages of product development and experimental measurement at a more advanced stage can provide a competitive advantage because of the time and costs being saved.

4.7. Biodiesel vs. food security

South Africa, like many other developing countries, is a net importer of oil. Many developing countries view the biofuels industry as a strategy to respond to threats of climate change, lessen their dependency on foreign oil and to stimulate and enhance agriculture, especially in rural areas (OFID, 2009a). SA, together with many Member States to the European Union, China, India, Indonesia, Thailand and the USA have all adopted policy measures and set targets for the development of biofuels. The intention by governments to pursue renewable energy in the form of biofuels is appealing and can be justified; however the social, economic and the environmental impact of such initiatives can be far reaching, especially if increased growth of biofuel crops if it has the potential to threaten the availability of adequate food supplies for humans by diverting arable land, water and other resources away from food and feed crops (OFID, 2009b).

Nolte (2007) found that agricultural land for the production of oilseeds for biodiesel production would have to be drastically increased to achieve commercial scale biodiesel production capacity. Nolte (2007) also suggested that despite the vast area needed to make biodiesel production a reality in SA, the utilisation of land previously cultivated for mainly maize production, could be used to cultivate sunflower and soybeans because the crops can be successfully grown in similar types of soil. Land for maize production has declined over the past decade as a result of an oversupply and associating weaker price for maize. Based on the “unused” maize production land, the potential to build a biodiesel economy in SA seems to be viable; however, other multifaceted factors such as government policies, tax incentives, biofuels production infrastructure and optimisation can influence the decision of not only government, but also investors to build a biodiesel economy in SA.

A robust and sustainable biofuels industry seeks to:

- Mitigate the threat of climate change by using renewable sources for transportation fuel production
- Ensure fuel energy security
- Limit rural to urban migration by developing rural agriculture

A robust and sustainable biofuels industry should not:

- Influence local food prices negatively (volatility of oilseed prices)

- Lead to food scarcity by increasing staple food prices
- Increase the price of transportation fuels that contain biodiesel
- Have a negative effect on the environmental and mechanical properties of transportation fuels that contain biodiesel

In order to achieve the benefits of a biofuels, and in particular a biodiesel industry in SA, the following strategy and policies should be considered (Worldwatch institute, 2006):

- Blending mandates
- Tax Incentives
- Government purchasing policies
- Support for biofuel-compatible infrastructure and technologies
- R&D (including crop research, conversion technology development, feedstock handling, etc.)
- Public education and outreach
- Reduction of counterproductive subsidies
- Investment Risk Reduction for Next-Generation Facilities
- Gradual reduction of supports as the market matures

4.8. Energy security

Growing food can be classified as another source of energy that needs to be produced and can be included in the larger framework of energy security. Growing population and economies require energy and the challenge for the future will be how to satisfy the need for energy, be it for vehicular transport, driving large industries, or powering urban and suburban homes. Like many countries, SA desires to be energy independent. Research into a more diverse energy portfolio for SA and developing alternatives to imported crude oil is the way forward and it may take many decades to become energy independent, but the research needs to be done today.

Nuclear, solar, wind, biological and even wastes to energy processes are in various stages of development currently to address our need for more energy. These alternatives to coal derived energy can only be successful if it is relatively cheap in terms of capital and operation investment, keeping in mind that diversification of energy resources is the key to sustainable generation. Just like a balanced and diverse investment portfolio is inherently safer than a single investment vehicle.

Potential environmental pollution, especially water contamination, is a concern since SA is a water scarce, semi-arid country. The potential to extract natural gas from shale rocks via hydraulic fracture method (also known as fracking) exist in the central part of SA, the Karoo, and the largely unknown environmental consequences of fracking in that region have not been researched in much detail. A governmental moratorium on exploration for shale gas in the Karoo was lifted in September 2012. It is the opinion of the author that fracking can be managed in an environmentally safe, responsible and sustainable manner with the latest techniques and necessary oversight in terms of the management of the process. The shale gas reserves in the Southern Karoo is estimated at 14 trillion cubic meters and by extracting the resource sustainably could change the energy landscape of SA and also southern Africa considerably. The necessary risk assessment and risk mitigation philosophies need to be applied to prevent catastrophic environmental harm since the cost of avoiding environmental harm is much less than managing the incident when it has occurred or remediation afterwards.

One aspect of the energy landscape that is sometimes easily overlooked is advances in energy efficiency. By implementing strategies, incentives, regulations and innovative

technologies to conserve energy, people's behaviour can be changed to be more energy efficient and in that way contribute to the energy independence of SA. The key is not one solution to the energy needs, but a multifaceted approach to the complex subject of sustainable energy security. The end of the hydrocarbon era of energy is not in sight, at least for the foreseeable future, but soon even hydrocarbon energy in the form of coal and crude oil will have to be re-evaluated as SA's major energy resource for economic and energy security.

-5-

Conclusions

1. It is concluded that the 2-D GC technique quantitatively and qualitatively identified the hydrocarbon constituents in the diesels. The relevance of using the 2-D GC technique was in identifying and quantifying the hydrocarbon breakdown products and being used in a mass balance to confirm the potential biological breakdown processes of the materials used in the present study.
2. The theoretical oxygen demand (ThOD) of the different experimental diesel blends using various blending materials and biodiesel, emphasised and confirmed the importance of calculating the ThOD for the respective blending materials when measuring the biodegradation rates.
3. The biodegradation hierarchy of Pitter and Chudoba (1990) in order of decreasing biodegradability: alkanes > branched alkanes > cyclo-alkanes > aromatic hydrocarbons, could be expanded to include FAME: FAME > alkanes > branched alkanes > cyclo-alkanes > aromatic hydrocarbons. The biochemical pathways identified for the biodegradation of all the diesels was enzyme-enhanced β -oxidation.
4. Biodiesel addition to crude-derived diesels to increase the density to within the current required specifications for diesels cannot be a reality in SA because of the underdeveloped biodiesel industry. To increase the density by using biodiesel to within the specification for GTL diesel, more than 27% biodiesel would be required, which is currently is not achievable from an economic perspective as well as governmental national strategy perspective. The addition of biodiesel as lubricity enhancer seems more plausible, because less than 5% would be required for petrodiesels.
5. The use of individual water accommodate fractions (WAF's) for each exposure concentration was effective to conduct ecotoxicity tests on sparingly soluble products, because the present research showed that acute toxicity effects were measured in the crustacean, algae and bacterial tests.
6. The WAF's of all diesels that were tested for ecotoxicity showed a general lack of acute

toxic effect, especially for the fish and crustaceans used. Although algal and bacterial tests showed an effect at most of the WAF loading rates, none were high enough to enable the calculation of a median effect loading rate (EL₅₀).

7. QSAR's, like EPI Suite, together with prediction models, like the FEEM, can be used to screen for ecotoxicity and biodegradability of hydrocarbons found in Petrodiesels. It was less applicable for the prediction of biodiesel constituents. The use of different cut-off values for the constituents of biodiesel could be developed in future research. The use of this combination enabled the present research into the potential toxicity of hydrocarbon mixtures to be conducted, especially since tests on individual constituents are impractical. QSAR's may provide a relatively cost-effective way to screen for potential environmental acceptability of such mixtures.
8. The contributors to the toxicity of mixtures of hydrocarbons found in diesels were evaluated and it appears that paraffins contribute more to the overall toxicity than previously thought and aromatics less.

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