Understanding the reproductive toxicity of gas oil substances: new Repro 1B self-classification and updated risk assessment

As part of Concawe's human health-related testing strategy, a scientific assessment has been undertaken concerning the reproductive and developmental toxicity of gas oil substances. This article summarises the findings of the study, focusing primarily on the OECD Test Guideline 422 results generated, in relation to a range of human health hazard and exposure scenarios. The study highlighted the need for further scientific inquiry and research directions.

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Introduction

Gas oils represent middle distillate hydrocarbon substances broadly utilised as diesel fuels, heating oils, lubricants, and a variety of other worker and consumer products. Categorised by Concawe as 'Other Gas Oils' (OGO), 'Vacuum Hydrotreated Gas Oils' (VHGO) and 'Straight-Run Gas Oils' (SRGO), these substances vary due to their refining processes and resultant chemical compositions. Gas oils predominantly encompass C10-C25 hydrocarbons, and are substances described as unknown or variable composition, complex reaction products, or biological materials (UVCBs). Given their extensive application across Europe (representing more than 350 million tonnes per year production or import in Europe), regulatory frameworks, notably the EU's REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) and CLP (classification, labelling and packaging) regulations, mandate comprehensive assessments to ascertain human health and safety, including the potential for reproductive and developmental toxicity. In this regard, this article explores recent advances and further implications, providing insights into the complexity of toxicological evaluation of these substances.

Gas oils and their complexities

Gas oils are, inherently, compositionally highly complex. They are categorised as UVCBs due to their variability arising from disparate crude oil sources and refining processes. Their complex chemical profile comprises thousands of distinct hydrocarbon constituents, including paraffins, olefins, naphthenic ring structures, and aromatic molecules with one to seven rings, all of which can have varying degrees of linear or ring (naphthenic) hydrocarbon groups branching from these structures.

Due to this diversity, advanced analytical techniques are essential for compositional elucidation. Two-dimensional gas chromatography (GCxGC) significantly improves analytical resolution, facilitating detailed hydrocarbon class identification and quantification by number of carbon atoms and molecular structure (paraffins, olefins, naphthenes, aromatics).[1] GCxGC provides what is known as a 'hydrocarbon space map' of a substance, that can be quantitatively used to evaluate individual substances and their categories. Concurrently, polycyclic aromatic compound (PAC)-2 analysis specifically quantifies 3-7 polyaromatic ring content, critical for evaluating toxicity (PAC-2 content has previously been hypothesised to associate with multiple toxicity pathways[2,3,4] and will be the main driver of reproductive and developmental toxicity).

Development of testing strategies and execution of OECD Test Guideline 422

By integrating the data from the analytical profiles of multiple samples of each substance within each Gas Oil category, candidate samples were identified to assess in vivo toxicity. The samples covered the worst case in the category PAC-2 content, complemented with other samples to cover the hydrocarbon space map of the categories as best as possible. These data serve as the initial basis of a confident read-across approach.

The OECD Test Guideline 422 (TG 422)[1] is employed extensively as a combined screening study to assess reproductive toxicity and developmental toxicity parameters, and to assess biological similarity. TG 422 outcomes facilitate establishing biological similarity, and enable biological read-across approaches to complement and verify the aforementioned analytical read-across. These are applied toward meeting higher-tier REACH requirements, such as 90-day repeated dose toxicity (RDT), prenatal developmental toxicity (PNDT) and extended one-generation reproductive toxicity (EOGRT) studies, and to reduce the number of these studies to be conducted. Importantly, the principal of the Concawe read-across hypothesis is that the samples chosen for in vivo analysis are representative of both the substance and category, such that the results from one sample are applicable to the other substances within the category.

As part of Concawe's human health-related testing programme for hydrocarbon substances, selected samples of all substances in the OGO, VHGO and SRGO categories were subject to TG 422 testing, through the oral exposure route by diet in rats. The dietary administration was chosen to achieve systemic exposure, and was supplemented by dermal studies to assess alternate exposure pathways. Within each of these categories, a 'worst-case' sample was identified based on 3 to 7 polyaromatic ring content as determined by the PAC-2 method, and other samples were selected to represent the overall hydrocarbon space map for a given category (see also the Concawe *Review* article on hydrocarbon space mapping[1]).

Results from the OECD TG 422 screening studies

The TG 422 studies have highlighted significant reproductive toxicological concerns for some tested samples. Observed effects include marked increases in post-implantation embryo losses, complete foetal lethality at elevated exposure levels, substantial reductions in litter sizes, and decreased foetal birth weights. Each worst-case sample from each of the three Gas Oil categories resulted in these adverse effects, and a fourth substance, not a worst-case by PAC-2, also had adverse reprotoxicity results (Figure 2). On the other hand, eight tested samples resulted in no adverse reproductive effects, and two samples generated indeterminant or equivocal results.The adverse results had a threshold of effect, i.e. in lower dose exposures of the same samples no reprotoxic effects were observed. These findings demonstrate clear, dose-dependent relationships, strengthening the evidence of reproductive hazards. Critical analysis of maternal toxicity indicators—such as altered body weights and reduced food consumption—provided strong evidence that reproductive outcomes are intrinsically linked to gas oil substances rather than secondary maternal toxicity effects, reinforcing the interpretation and further classification determinations.

[1] Test No. 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test. https://doi.org/10.1787/9789264242715-en

Figure 2: Selected OECD TG 422 testing results for gas oil samples

EC number	265-078-2	265-043-1	270-676-1	270-671-4	265-148-2	269-822-7	265-182-8	272-341-5	265-049-4	265-044-7	265-183-3	270-673-5	265-059-9	272-817-2
CAS number	64741-77-7	64741-43-1	68476-34-6	68476-30-2	64742-46-7	68334-30-5	64742-79-6	68814-87-9	64741-49-7	64741-44-2	64742-80-9	68476-31-3	64741-58-8	68915-96-8
PAH/sample no.	VHGO_S692	SRGO_S686	VHGO_S668	VHGO_S760	OGO_S726	VHGO_S777	OGO_S712	SRGO_S836	VHGO_S721	SRGO_S795	OGO_S809	VHGO_S845	VHGO_S796	SRGO_S715
1-ring	0.04	0.19	0.57	0.59	0.09	0.42	0.58	0.26	0.3	0.05	0.34	0.19	0.07	0.08
2-ring	0.46	0.86	1.94	1.36	1.42	1.74	2.28	4.42	7.2	1.08	4.45	1.01	1.75	1.25
3-ring	0.06	0.13	0.22	0.24	0.29	0.47	0.51	1.85	2.5	3.48	3.36	3.46	4.76	2.99
4-ring	0	0.01	0	0	0	0	0	0	0	0.09	0.25	0.19	0.42	3.24
5-ring	0	0	0	0	0	0	0	0	0	0	0	0	0	0.75
6-ring	0	0	0	0	0	0	0	0	0	0	0	0	0	0
≥ 7-ring	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total 3+ PAH (PAC-2)	0.06	0.14	0.22	0.24	0.29	0.47	0.51	1.85	2.5	3.57	3.61	3.65	5.18	6.98

R positive
equivocal
negative

	Dose	S692	S686	S668	S760	S726	S777	S712	S836	S721	S795	S809	S845	S796	S715	
Mean no. of implantations	0	17.7	17.4	15.4	15.7	12.3	16.7	17.3	17.2	16.8	17	16.1	17.8	16.8	15.6	
	100	15.1	17	15.4	15.4	15.8	16.4	15.7	16.1	15.6	17.1	16.7	15.4	16	16.2	
	300	17.4	18.1	14.3	17	13	16	16	16.5	14.8*	15.9	15.3	15.3	13.9*	15.6	
	750	17	15.1*	14.9	13.9	12.3	15.4	14.4*	13.4*	14.4*	15.5	13.6*	12.9*	13.3*	11.4*	Reduced by > 20–25%
	1,000						15.8	15*				13.3*		13.0*	12.0*	
Mean % post-implantation loss		23.3	16.3	14.1	26.1	23.5	15.1	14.3	12.6	10.9	27.4	23.3	14.9	14.7	12	
		12.5	19.6	11.6	18.4	17	7.1	8.7	12.6	15.9	11.1	26.4	13.4	12.7	15.7	
		25.8	11	8.6	12.8	8.1	9.5	12	11.8	10.3	19.2	33.6	9.3	31.1	16.2	
		23	10.1	16.1	16.7	28	20.1	25.1	13.6	35.3	19.3	50.8	14.6	44.1	43.7*	Observed > 30%
							12.3	15.9				100		45.3	75*	
Mean litter size at PND 0		13.6	14.6	14.7	11.6	9.6	14.2	14.8	14.9	14.9	14.3	12.6	15.2	14.5	13.9	
		13.4	13.7	13.7	12.8	13	15.2	14.4	14	13.1	15.1	12.8	13.4	13.9	13.7	
		14.3	16	13.9	14.8	13.3	14.4	14	14.4	13.3	12.8	10.2	13.8	10.6	13.1	
		14	13.6	12.6	11.7	10.2	12.2	10.7*	11.6	9.3*	12.3	6	11*	9.4*	6.3*	Reduced by > 20–25%
							13.9	12.6				0		9.1*	5.8*	
Mean fetal weight at birth		6.5	6.6	6.4	6.6	6.3	6.5	6.5	7.0	6.7	6.8	6.9	6.6	5.0	6.7	
		6.7	6.9	6.5	6.7	6.5	6.6	6.5	6.7	6.7	6.8	6.8	7.1	6.5*	6.6	
		6.6	6.3	6.6	6.6	6.7	6.3	6.4	6.5	6.3	6.8	6.8	6.6	6.7*	6.3	
		6.4	6.4	6.8	6.5	6.8	6.9	7.0	6.9	6.7	6.5	5.8	6.7	6.8*	6.5	
							6.4	6.4				0		6.2	5.5	Reduced by > 5–10%

Reproductive toxicity category 1B (Repro 1B) self-classification and update of hazard characterisation

After extensive evaluation by Concawe's Health Management Group (HMG), supplemented by independent external expert consultation, gas oils were determined to warrant a Repro 1B (H360FD: 'May damage fertility; May damage the unborn child') self-classification under the EU CLP regulation. This classification indicates definitive animal-based evidence demonstrating potential adverse reproductive impacts on humans. This classification was substantiated by the reproductive toxicity outcomes across multiple TG 422 studies in all Gas Oil categories, and was corroborated by supporting data from a PNDT rat study, aligning with the European Chemicals Agency (ECHA) stance on the data generated. Though the adverse effects were only observed for some tested samples, the new Repro 1B self-classification has been applied to all substances within the categories, given the aforementioned read-across principles and based on the worst-case sample testing outcome, even if the adverse toxicity effects were not observed in other samples tested within the categories.

Category 1 Carcinogen, Mutagen or Reprotoxin (CMR) classification has implications for uses of the gas oil substances. However, importantly, per the EU's CMR Directive 2022/431/EC, the H360 classification specifically has a call-out regarding thresholds: '*For most reprotoxic substances, it is scientifically possible to identify levels below which exposure would not lead to adverse health effects. The exposure minimization requirements laid down in Directive 2004/37/EC should apply only to reprotoxic substances for which it is not possible to identify a safe level of exposure and which are identified as "non-threshold" in the notation column of the Annex III to Directive 2004/37/EC. With regard to all other reprotoxic substances, employers should ensure that the risk related to the exposure of workers is reduced to a minimum.*' As the TG 422 results indicated a threshold of effect, systemic long-term Derived No-Effect Levels (DNELs) could be determined (based on No Observed Adverse-Effect Levels (NOAELs) from TG 422) and indicating a safe level of exposure below which there is no risk to workers' or consumers' health (see Table 1). These DNELs have undergone expert review by the HMG (numbers in bold) and they are generally lower than the previous DNELs (numbers in parentheses) of these categories, and in the case of VHGO, they are much lower.

Table 1: Worker and general population DNELs for Gas Oil categories

	Worker DNELs Long term = 8-hour time weighted average Acute = 15 minutes			**General population DNELs** Long term = 24 hour Acute = event			
	Inhalation systemic long-term (mg/m^3)	**Inhalation systemic acute (mg/m^3)**	**Dermal systemic long-term (mg/kg/day)**	**Inhalation systemic long-term (mg/m^3)**	**Inhalation systemic acute (mg/m^3)**	**Dermal systemic long-term (mg/kg/day)**	**Oral systemic long-term (mg/kg/day)**
OGO	**16.46** (16.40)	5,003	2.91	**3.48** (4.85)	3,002	1.25	**2.50** (1.25)
VHGO	**5.49** (68.34)	4,288	2.91	**1.16** (20.22)	2,573	1.25	**0.83** (1.25)
SRGO	**5.49** (16.40)	1,501	2.91	**1.16** (4.85)	900	1.25	**0.83** (1.25)

Update of exposure scenarios and risk assessment

Safe use is determined by the measured or modelled exposure to a substance being less than the DNEL (i.e. a risk characterisation ratio (RCR) < 1). Previously, gas oil safe use was primarily[2] evaluated and confirmed using the conservative Tier 1 exposure assessment model ECETOC TRA v3.1[5] for both inhalation and dermal exposures. This model is very generalised and makes many conservative assumptions and estimates. However, the decrease in DNELs combined with the revision of TRA to v3.2 to include a more conservative inhalation model (i.e. overestimation of inhalation exposure) indicated exposures higher than the DNEL for almost all uses. Therefore, Concawe has launched an effort to refine exposure assessments to more accurately estimate the inhalation exposure that occurs in gas oil uses.

[2] Importantly, Concawe has conducted some measured data campaigns for gas oils that have been invaluable in the evaluation of their risk (Concawe reports 1/06 and 14/14).

This effort involved the use of Concawe Report no. 1/06, *Human exposure information for EU substance risk assessment of gas oils*.[6] This report contains, in specific detail, a wide variety of industrial and professional jobs and tasks associated with the manufacture, distribution and retail use of gas oils. These fuel-related uses constitute more than 99.9% of the registered tonnage for these substances. These specific and relevant tasks were used to develop exposure scenarios that map to the existing described fuel-related worker uses; these exposure scenarios were then integrated into a higher tier inhalation model, the Advanced REACH Tool (ART) v1.5.[7] Of note, dermal and consumer exposure assessments are still performed using TRA v3.2 as the ART model is currently not fit for purpose for these particular assessments.

Inhalation exposure is driven by a composite of two general airborne entities: aerosols and vapour. Previous research by Concawe has shown that gas oils belong to the group of 'semi-volatile' hydrocarbon substances, i.e. when released to air (for example, as a result of product transfer activity) the resulting stable atmosphere contains vapour and mist (the latter is also called aerosol). The gas oil fraction that can give rise to vapour levels in air consists of the product constituents with individual vapour pressure greater than 10 Pascal (Pa). The complementary fraction, i.e. constituents with individual pure substance vapour pressures below 10 Pa, are assumed to form aerosol (minute droplets) when released to air. The cut-off at 10 Pa between a vapour-generating and aerosol-generating substance is implemented in the ART model and was adopted in the Concawe method for occupational inhalation exposure estimation.

As previously mentioned, GCxGC data were collected as a part of the Concawe Substance Identity Management Group (SIMG) efforts from samples across all substances of each category to develop hydrocarbon space maps. These maps provide median weight percentages in the category for individual hydrocarbon blocks (HCBs) according to carbon number and hydrocarbon chemical class. The vapour pressure of each HCB was estimated by applying boundary layer theory to adjust the estimated air releases.[8] HCBs with vapour pressures ≥ 10 Pa at 25°C constitute the vapour fraction of a substance. This cut-off was based on the definition in the ART user guide. The percent composition of the vapour ART assessment entity was determined by summing the normalised median substance HCBs weight percents (wt%). HCB wt% were converted to mole fractions by dividing by the estimated molecular weights of the HCB. The vapour pressure of the vapour ART assessment entity was determined via Raoult's law where the vapour pressure of a mixture is calculated by summing the products of (mole fraction of a constituent (here HCB)) multiplied by (vapour pressure of that constituent) for all the constituents of that mixture.

It was determined that the median vapour component vapour pressures are 255, 222 and 168 Pa, and mole fractions are 36.6%, 30.2% and 17.5% for VHGO, OGO and SRGO, respectively (thus, VHGO is the worst-case substance for DNEL and volatility).

Importantly, all Gas Oil categories also have many registered non-fuel uses (e.g. lubricants, coatings, oil and gas drilling, and road construction). While these uses constitute < 0.1% of registered tonnage, they represent a wide variety of uses (up to 21 per category) with many contributing scenarios (up to 15) for each use. Creating new unique exposure scenarios to be run in ART was a task too large for Concawe's staff and expertise. As such, these non-fuel uses have been assessed, with some input from registrants and trade associations, with regard to the gas oil composition of the substances used using TRA v3.2.

Risk management measures (RMMs) have been applied to achieve safe use according to HMG's hierarchy of RMMs, prioritising ventilation and time management measures.

Implications for safe use

Using these scientifically-developed exposure scenarios with the measured compositional data-derived vapour pressures, all fuel uses were assessed in ART for inhalation exposure.[3]

Importantly, all fuel-related uses have been determined as safe and supported without the need for additional RMMs beyond those described in the existing job tasks from Concawe Report 1/06.[6] This covers work at refineries, formulation and storage sites, distribution terminals, distribution drivers,[4] and refuelling tasks including full-service service station attendants and mechanics. Notably, to mitigate worker dermal exposure, all tasks now require the use of chemical-resistant gloves with one exception: service station attendants for whom measured data are available—see Concawe Report no. 14/14.[9]

Consumer use of gas oils as fuel (for refuelling automotive diesel engines, garden equipment and recreational vehicles) was also assessed as safe when using the available REACH modelling tools, as well as when using the limited inhalation and dermal exposure measurement data available from previous Concawe projects.

Most non-fuel uses relied on TRA v3.2 modelling and have achieved safe use for almost all uses. However, to achieve safe use, many stringent RMMs have been implemented across almost every exposure scenario. This has involved: reducing the percentage (from 100%, as reflected by industry association and/or registrant input) of gas oil in the use; increasing general room ventilation to minimally 3–5 air changes per hour; implementing local exhaust ventilation; and/or in a few cases (e.g. manual spraying) requiring worker respirators.

[3] Fuel assessments were made at 100% gas oil substance. This may not reflect the real-world product considering additives and renewable component content which varies across EU Member States.

[4] VHGO road tanker (distribution) driving is the highest exposed task (RCR = 0.953). It should be noted that the exposure scenario is based on top loading which was prevalent at the time of Concawe report 1/06. Bottom loading is considered standard practice at present, which significantly reduces inhalation exposure, and as such this RCR value is likely a conservative overestimate.

Regardless of RMM interventions, some non-fuel exposure scenarios cannot achieve safe use. In particular, the use of SRGO and VHGO as a drilling mud in oil and gas field drilling operations is no longer supported and is advised against. Additionally, a few other contributing activities have required reformulations (reduction of the gas oil component), e.g. use in coatings and use in lubricants for professional and consumer contributing activities.

Future directions on exposure modelling

It should be noted that a major component of modelled gas oil exposure comes from the results of TRA v3.2 dermal exposure modelling. This model assumes that the entirety of a substance is instantly absorbed through the skin, which is known not to be the case for gas oils. However, it is the only available assessment model that is readily applied to the uses and substances in the Concawe portfolio. The RCR contribution from this TRA v3.2 modelled dermal exposure is generally 0.471, in other words nearly half the allowable exposure.

The Concawe portfolio would greatly benefit from the development of higher-tier dermal modelling platforms (e.g. the dermal module in ART v1.5). Additionally, measured data for dermal exposure is sparse for gas oil (and almost all Concawe substance) uses. The Concawe portfolio would equally benefit from projects that would support the gathering and/or generation of additional measured dermal exposure data.

Conclusion

Recent advances in the reproductive toxicity assessment of gas oils have impacted the classification and risk management frameworks. Continuous scientific inquiry and adaptive adjustments are imperative for ensuring human health protection, maintaining regulatory compliance, and supporting the sustainable use of gas oil substances. Importantly, this article is associated with human health hazard and exposure scenarios only. Other regulatory implications addressing, for example, labelling, and safe transport, transfer and storage, were also assessed but are beyond the scope of this article.

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