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Health and environmental risk assessment of microbial cleaning products

Opinion of the Panel on Microbial Ecology of the Norwegian Scientific Committee for Food Safety

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Risk assessment on Health and environmental risk assessment of microbial cleaning products

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Health and environmental risk assessment of microbial cleaning products

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(Authors in alphabetical order after chair of the working group)

Assessed and approved

The opinion has been assessed and approved by the Panel on Microbial Ecology. Members of the panel are: Ida Skaar (chair), Tor Gjøen, Jacques Godfroid, Anders Jelmert, Jörn Klein, Arinze Okoli, Arne Tronsmo, and Bjørnar Ytrehus.

(Panel members in alphabetical order after chair of the panel)

Acknowledgement

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) appointed a working group consisting of VKM members to answer the request from the Norwegian Environment Agency. Project leader from the VKM secretariat has been Nana Asare. The members of the working group Ida Skaar, Anders Jelmert, Bjørnar Ytrehus, Jacques Godfroid are acknowledged for their valuable work on this opinion. The Panel on Microbial Ecology is acknowledged for comments and views on this opinion.

Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

In 2015, The Norwegian Environment Agency requested the Norwegian Scientific Committee for Food Safety (VKM) to provide a scientific assessment of the information requirements laid down in the declaration form for the Regulation on microbial products and its guidelines, if these are sufficient to conduct a health and environmental risk assessment of the use of microbial cleaning products in Norway. VKM appointed a working group consisting of members of the Panel on Microbial Ecology. The Panel on Microbial Ecology has reviewed and revised the draft prepared by the working group, and the assessment has been adopted.

Based on scientific assessment of the information requirements laid down in the declaration form of the Regulation on microbial products, the VKM Panel recommends that the information requirements in their current form should be revised to facilitate health and environmental risk assessment of the use of microbial cleaning products in Norway.

There seems to be a general lack of accuracy when it comes to specification of the microbial content and concentrations (metabolically active vs. inactivated or dead cells) in the product. Without proper taxonomic classification, no meaningful risk assessment is feasible. The taxonomic affiliation of the organisms present in the product should be specified to at least species, preferably strain level.

The declaration should in our opinion not necessarily rely on specific methods, as long as the methods described are scientifically adequate. However, the identification should be based on new molecular methods, for e.g. the potential role of the microorganism in the product acting as a pathogen or an allergen, its association to intestinal dysbiosis or genes coding for antibiotic resistance can be identified. Rather than specifying a list of specific antibiotics one should employ generic classes of antibiotics as stipulated in the Nordic Ecolabelling guidelines. The Panel recommends a multiphasic approach to future assessments, as this allows for the implementation of current and most effective methods as they are developed and verified.

There seems to be lack of emphasis on environmental impacts, especially on the potential for persistence and spread in the environment (terrestrial or aquatic), the potential for pathogenic effects on domestic or wild vertebrates, arthropods or plants. Furthermore, there is little emphasis on the effects with increased use and accumulation, persistence and spread, both indoor, in terrestrial and aquatic environments and on long-term effects on the microbial community.

The state (living, dead, inactivated) and form (vegetative, viable spores (bacteria and fungi) or cysts (protozoans)) of the microorganism present should be specified. If the product contains organisms that form endospores, spores or cysts, procedures for activation of the spores or cysts and for further cultivation should be described. This makes it possible to test whether the spores or cysts are not viable. The method employed for eventual inactivation

or sterilization such as heat or chemical treatment, radiation, or dose, and the exposure time and concentration of the micro- organism should be described. Information on how the product has been tested to ensure that it does not contain live microorganisms is also required.

The declaration should provide information about the procedures and quality controls securing a product without contaminations, pathogens, or known relevant virulence or resistance factors that may increase health or environmental risks. The safety reassurances provided by producers of microbial cleaning products, should also cover properties related to allergenicity, sensitization, plant pathogenicity and environmental impacts. How the microbes in the product and their pathogenic properties develop with time through and after shelf life should also be described.

In our opinion a declaration should include information about intended use and instructions for use, if specific precautions (personal protection, waste, containers etc.) need to be taken. Furthermore, information relating to user groups should be provided; for example if the product is suitable for use in certain settings and environments such as healthcare institutions, food facilities, and areas with vulnerable people (immunocompromised, infants, elderly, pregnant women etc.) or production animals.

The term "Environmental Damage" is not sufficiently defined. What kind of shift in the microbial community and local community can be expected in the receiving environment, especially if exposure is chronic and frequent? The document focuses only on the introduction of foreign genes into the ecosystem. The environment can also be permanently altered (or damaged) if the introduction of the new organisms results in the extinction of the naturally existing closely related species. In addition, metabolic products that might affect resident microbial communities could be valuable information.

A re-evaluation of current national and international regulatory and policy frameworks may be necessary. This can include an evaluation of the most appropriate instruments (e.g. product declaration forms, regulations, standards, codes of practice, etc.) to use for strengthening these frameworks to mitigate risks to human health and the environment.

Key words: VKM, (benefit and) risk assessment, Norwegian Scientific Committee for Food Safety, Norwegian Environment Agency, microbial cleaning products, microorganisms.

Sammendrag på norsk

Vitenskapskomiteen for mattrygghet (VKM) har på oppdrag fra Miljødirektoratet vurdert om dagens krav til informasjon om rengjøringsprodukter som inneholder mikroorganismer gir et godt nok grunnlag til å foreta helse- og miljørisikovurderinger av produktene.

Den som importerer, produserer eller omsetter mikrobiologiske produkter i Norge er pålagt å merke og deklarere produktene i Produktregisteret i henhold til et eget deklarasjonsskjema.

Miljødirektoratet har bedt om en faglig vurdering av hvilken informasjon direktoratet skal etterspørre når et produkt skal vurderes med hensyn til helse- og miljørisiko i Norge.

Hvis dagens krav ikke er gode nok, ble VKM bedt om å vurdere hvilke krav som bør stilles.

VKMs faggruppe for mikrobiell økologi har gjennomgått og vurdert dagens krav til informasjon i deklarasjonsskjemaet «Forskrift om mikrobiologiske produkter». VKM mener at dagens krav ikke gir tilstrekkelig grunnlag for å gjennomføre en vurdering av helse- og miljørisiko knyttet til bruk av mikrobiologiske rengjøringsprodukter i Norge. VKM anbefaler at kravene til informasjon revideres.

VKM mener det er en generell mangel på presisjon i spesifiseringen av det mikrobielle innholdet og konsentrasjonen av mikroorganismer (metabolsk aktive kontra passive eller døde celler) i produktet. Uten en grundig klassifisering av mikroorganismene, er det ikke mulig å gjennomføre risikovurdering av produktet. Tilhørigheten til mikroorganismen i produktet bør spesifiseres til minst artsnivå, helst også stammenivå.

Etter faggruppens mening, bør ikke krav til identifisering avhenge av spesifikke metoder så lenge de generelle metodene som benyttes er vitenskapelig dekkende. Identifiseringen bør baseres på nye molekylære metoder, f.eks. den aktuelle mikrobens potensiale som patogen, allergen eller toksigen, eventuelle assosiasjoner med mikrobiell ubalanse i kroppen eller gener som koder for antibiotikaresistens kan identifiseres. I stedet for å angi spesifikke antibiotika, bør det angis generiske klasser av antibiotika på samme måte som i Svanemerkets retningslinjer. Det bør tilstrebes en bred metodisk tilnærming. Det vil muliggjøre at nye og mer effektive analyser eller evalueringsmetoder kan tas i bruk så snart de er publisert og validert.

Det ser også ut til at det er lagt for lite vekt på miljøpåvirkninger. Det gjelder spesielt potensialet for overlevelse og spredning i miljøet, både til lands og til vanns, og til å påføre ville eller domestiserte virveldyr, leddyr eller planter sykdom. Videre er det lagt liten vekt på effektene ved økt bruk og akkumulering, persistens og spredning både innendørs og i jord og vann, og langtidseffekter på det mikrobielle miljøet.

Den aktuelle mikrobens stadium (levende, død, inaktivert) og form (vegetative, levedyktige sporer (bakterier og sopp) eller cyster (protozoer) bør spesifiseres. Dersom produktet

inneholder organismer som danner endosporer, sporer eller cyster, bør prosedyrer for aktivering og videre kultivering beskrives. Dette gjør det mulig å teste om sporer eller cyster har blitt drept. Metoden som er benyttet til eventuell inaktivering eller sterilisering som varmebehandling, stråling, kjemisk, eller dose, og eksponeringstid og konsentrasjonen av den aktuelle mikroorganismen, bør beskrives. Det er også nødvendig med informasjon om hvordan produktet har blitt testet for å sikre at det ikke inneholder levende mikroorganismer.

Deklarasjonen bør også gi informasjon om prosedyrer og kvalitetskontroller som sikrer at produktet ikke inneholder kontaminanter, patogener eller kjente relevante virulens- eller resistensfaktorer som kan øke risiko for helse og miljø. Sikkerheten som er angitt av produsenter av mikrobielle rengjøringsprodukter, bør også dekke egenskaper relatert til allergenisitet, følsomhetsreaksjoner, plantepatogenitet og miljøpåvirkning. Det bør også beskrives hvordan mikrobene i produktet og deres sykdomsframkallende egenskaper utvikles under og etter lagring over tid.

VKM mener en deklarasjon bør inneholde informasjon om bruksområder og instruksjoner for bruk dersom det er behov for spesielle forholdsregler (personlig beskyttelsesutstyr, avfall, emballasje osv.).

Videre bør deklarasjonen inneholde informasjon relatert til brukergrupper. Det kan for eksempel være om produktet kan brukes i spesielle sammenhenger og miljø som helseinstitusjoner, matproduksjonslokaler, lokaler for sårbare grupper (personer med nedsatt immunforsvar, barn, eldre, gravide osv.) eller lokaler for produksjonsdyr.

Begrepet "miljøskade" er ikke tilstrekkelig definert i dagens forskrift. Hva slags endring av mikrobielle samfunn og miljøet lokalt kan for eksempel forventes i det eksponerte miljøet dersom eksponeringen er kronisk og frekvent? Dokumentet fokuserer kun på introduksjonen av fremmede gener i økosystemet. Miljøet kan også bli permanent påvirket eller skadet dersom introduksjon av en nye organisme resulterer i utryddelse av nært beslektede arter som finnes naturlig i miljøet. I tillegg kan det være verdifullt å få informasjon om metabolske produkter som kan påvirke eksisterende mikrobielle samfunn.

En revurdering av gjeldende nasjonale og internasjonale regulatoriske rammeverk kan være nødvendig. Dette kan omfatte en evaluering av de mest hensiktsmessige virkemidlene for å styrke disse rammeverkene og redusere risiko for human helse og miljøet. Eksempler på virkemidler kan være produkterklæringer, forskrifter, standarder, og regler for god praksis.

Abbreviations

AB	Antibiotic
CFU	Colony-forming units
CLP	Classification, Labelling and Packaging
EC	European Commission
EEC	European Economic Community
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ISO	International Organization for Standardization
NIH	National Institutes of Health
OECD	Organization for Economic Co-operation and Development
SH	Sulphur and Hydrogen
YOPI	young, old, pregnant, immune compromised

Background as provided by the Norwegian Environment Agency

Introduction

Cleaning products containing microorganisms as an active substance have become more common in the market. To date, there are no common international regulations or quality standards regulating the production and use of microbial cleaners. A lack of common quality standards is a challenge for both industry and regulators. Several organizations involved in the eco-certification of products have included requirements for microbial cleaning products. Among others, the Nordic ecolabel, the Swan, describes their requirements for documentation and information of microbial cleaners in their criteria document for cleaning products.

Regulatory background

In Norway microbiological products are regulated as any other product on the market under the Act relating to control of products and consumer services (the Product Control Act of 6 November 1976), and under a separate regulation of 22 January 1998 no. 93 relating to the declaration and labelling of microbiological products (Regulations on microbiological products). The purpose of the regulation on microbiological products is to prevent microorganisms in microbiological products from causing damage to health or adverse environmental effects such as disruption of ecosystems, pollution, or waste.
According to the regulation, any person that manufactures or imports microbiological products or places them on the market in Norway has a duty to declare any information necessary for an assessment of the risk the product poses to human health or possible negative environmental effects. The information is to be given in a declaration form (cf. appendix of the regulation) and amongst others include a description of the product and its composition, area of application, mode of use, and degradation products, antibiotic resistance and any pathogenic properties of the microorganisms. The guidelines to the regulations provide detailed description of the type of information and which documentation is required to satisfactorily declare the product. The information provided shall give the authorities a basis to assess the health and environmental risks associated with the use of the products.

In the EU, microbial cleaners fall partly under EU Directive 2000/54/EC "on the protection of workers from risks related to exposure two biological agents at work." This directive regulates employer obligations and employee rights in a work environment where workers are exposed to biological agents. It has been under consideration whether Detergent Regulation 648/2004 should be updated with respect to microbial cleaning products, but this

is currently not the case. Standards for these products are also not included under the EU Ecolabel.

The Norwegian Environment Agency considers that there is a need to update the information and documentation requested from manufacturers of microbial cleaning products, to be able to sufficiently risk assess microbial cleaning products.

Terms of reference as provided by the Norwegian Environment Agency

The Norwegian Environment Agency therefore requests VKM to:

1) provide a scientific assessment of the information requirements laid down in the declaration form of the Regulation of Microbial products (and as further specified in the guidelines to the Regulation) and if these are sufficient to conduct a health and environmental risk assessment of the use of microbial cleaning products in Norway

2) if no under assignment 1) provide a scientific assessment of updated information requirements that producers and importers must / should meet, that can be used as a basis for the risk assessment of health and environmental risks associated with microbial cleaning products in Norway

It must be considered whether the proposed information requirements can be obtained using internationally recognized methods available today. An evaluation of the criteria laid down in the Nordic Swan Ecolabel could be relevant in the eventual case that new information criteria are recommended. The assessment must include an evaluation of whether there is sufficient knowledge to carry out risk assessments of microbial cleaners in Norway. Critical knowledge gaps for the risk assessment of microorganisms must be included.

The following is not included in the assignment: Assessment of genetically modified microorganisms (GMMO) is not included in the assignment as these are regulated under other legislation and procedures.

Assessment

1 Literature

1.1 Background literature provided by the Norwegian Environment Agency

Norwegian Regulation on Microbial products:
https://lovdata.no/dokument/SF/forskrift/1998-01-22-93
https://www.regjeringen.no/en/dokumenter/declaration-and-labelling-of-microbiolog/id440456/

Klade, M & Spök, A (2009), Environmental, health and legal aspects of cleaners containing living microbes as active ingridients, IFZ Electric Working papers #3.
Available from: www.ifz.tugraz.at/publikationen/electronic-working-papers

OECD (2015) *Biosafety and the Environmental Uses of Micro-Organisms*: Conference Proceedings, OECD Publishing

Nordisk Miljømerking, Svanemerking av rengjøringsmidler, versjon 5.2, 25.september 2014.
Available from:
http://www.svanemerket.no/svanenskrav/rengjoringsprodukter/rengjoringsmidler/

1.2 Literature search

The following search terms and combinations thereof were employed:
microbial/microbiological cleaning, cleaning products, green cleaning, ecolabelling, bacterial identification, bacteria as allergens & "Review".

Sources: Pubmed, Google scholar

Search results were analysed for those that were of relevance. Each working group member performed relevance screening independently. The reference lists in selected citations were further assessed to identify additional articles that were of relevance.

2 Introduction

The "new green revolution" is gaining traction as awareness increases on the environmental impacts and health risks resulting from the use of chemicals. This new revolution is not limited to the agricultural and food industry, but is wide-ranging and cuts across many sectors and activities. The manufacturing of cleaning products is one of the industries affected by this trend. The general population as well as institutions in the healthcare sector are increasingly embracing what is now broadly referred to as "green cleaning" (Quan et al., 2011). The use of microorganisms in cleaning products is a part of this development.

Manufactures and distributors often make claims of eco/environmental-friendliness since microbes already abound in nature. Thus, microbial-based cleaning products are becoming popular as consumers are in search of milder alternatives than the more or less inherently toxic and often highly corrosive nature of chemicals in conventional cleaners. Moreover, microorganisms used in cleaners have been found to be efficient in preventing sediment formation in sewage pipes/plumbing, drains and grease traps (Spök and Klade, 2009). Producers frequently make claims of economical dosages resulting from high dilutions of concentrated products before application, and thereby prospects for cost reduction.

Similar to other types of products containing microbes, microbial-based cleaning products harness the capability of living microorganisms to produce extracellular enzymes such as cellulases, proteases and ureases. These enzymes can degrade high molecular weight compounds often associated with dirt/"soil" and effectively mitigate problematic odours by further metabolism of intermediates from these processes. Nitrifying and sulphur-oxidizing bacteria can convert ammonia and thiols (-SH containing compounds); often intermediates of degradation processes characterized by strong pungent or foul smells, to odourless nitrate and sulphate alternatives, respectively (Friedrich, 1998; Kampschreur et al., 2006; Kuenen et al., 1985). Thus, microbial action usually aims at controlling odour in addition to supporting the cleaning action of detergents. Unlike most conventional cleaners that mask bad odours with fragrances or perfumes that lose their effect shortly, some producers claim microbial cleaners eliminate bad odours and simultaneously prevent them from reoccurring.

Most of the cleaning formulations employ spores or spore-forming bacteria that are viable for up to a year after application (OECD, 2015; Spök and Klade, 2009) and as such, it is not surprising that manufactures claim long-term effects. Some microorganisms are capable of directly inhibiting the growth of other unwanted microbes by changing certain factors in the microenvironment such as pH, hindering re-colonization of such microbes, and seal off the surface.

Although there is concern for association between the use of microbial-based cleaning products and potential respiratory sensitization, in case of chronic exposure, this is more true for the use of microbial enzymes employed in consumer products and not the microorganisms in particular (Martel et al., 2010). Interestingly, some producers claim microbial cleaners reduce allergenic reactions by out-competing and hence mitigating mites, moulds and other allergenic agents. Notably, some mould species like *Aspergillus oryzae* employed in some microbial-based cleaning products may possess allergenic properties (Spök and Klade, 2009).

There is limited information available to the public, insufficient scientific knowledge and lack of standardized regulations to enable this promising industrial field to grow in a sustainable manner (OECD, 2015; Thomas and Versteeg, 2013). Presently, most organizations that deal with standards and certification of environmentally friendly products and services have included criteria for microorganisms in cleaning products. One such organization is the Nordic Ecolabelling/Swan popularly known as "Svanemerket" in the Scandinavian countries. The Nordic swan is the official sustainable ecolabel for the Nordic countries, a voluntary license system, introduced by the Council of Ministers. Its American counterpart is Green Seal, co-founder of Global Ecolabelling Net.

Microbial products are regulated under the Act relating to control of products and consumer services (the Product Control Act of 6 November 1976), as any other product on the market in Norway. Additionally, a separate regulation regarding the declaration and labelling of microbiological products of 22 January 1998 no. 93 (Regulations on microbiological products) is also in force, with the primary aim to prevent microorganisms in microbiological products and technologies from causing adverse effects to biological health, the physical environmental and ecosystems. This regulation impose on importers, distributors as well as manufacturers of microbiological products in Norway to declare any information necessary for assessing the risk the product poses to human health or potential negative environmental effects. The guidelines to the regulations provide detailed description of the required information and documentation needed to declare a product in a satisfactory manner. This information provides the authorities with the basis to assess the health and environmental risks associated with the use of such products. The Norwegian Environment Agency considers that it is needful to update the information and documentation requested from manufacturers, importers and distributors of microbial cleaning products, to be able to risk assess microbial cleaning products effectively.

Within the EU-harmonized legislation, microbial cleaners fall partly under EU Directive 2000/54/EC "on the protection of workers from risks regarding exposure to biological agents at work." This directive regulates employer obligations and employee rights in a work environment where workers are exposed to biological agents. However, the risk group scheme employed is limited to human pathogenicity and relevant factors like potential allergenic effects or sensitizing properties of microorganisms, among others are not considered. Additionally, the risk of microbes on animals, plants, ecosystems and the physical environment are not assessed.

Varying microorganisms and combinations of these microbes with other ingredients like enzymes and in some cases, chemicals are employed in microbial-based cleaning products (OECD, 2015; Spök and Klade, 2009). Members of the genus *Bacillus, Bifidobacterium, Lactobacillus, Rhodopseudomonas* and *Saccharomyces* are commonly used (Spök and Klade, 2009). Other bacterial genera represented in these products include *Actinobacter, Alcaligenes, Arthrobacter* and *Rhodobacter* (OECD, 2015). Another fungal genus aside *Saccharomyces* is *Candida*, both of which have been observed to be effective in the biodegradation of a variety of hazardous chemicals (Harms et al., 2011; Liu et al., 2011). Some of these microbes are considered beneficial whereas others have a long history of safe use in other industrial sectors like refineries, brewing, winemaking and baking (OECD, 2015). Spores are preferred over vegetative cells as they prolong the shelf life of the product for up to a year (OECD, 2015; Spök and Klade, 2009). A product survey conducted by Spök and Klade disclosed that most producers considered the precise identity of microbes such as species and strain as confidential and as such withheld information (Spök and Klade, 2009). A Canadian survey reports similar findings (OECD, 2015). Problematic issues related to detailed information on formulations as well as use of products have been noted. Indications of inconsistencies in quality control and/or assurance during production of microbes and end products that relates to proper taxonomy have been observed (OECD, 2015; Spök and Klade, 2009).

Accurate taxonomic identification is a key step in risk assessment. The reliability of this step remains uncertain if the producers are allowed to provide information with poor taxonomic resolution.

Uncertainties exist in the extent of human exposure, long-term impacts on the environment as the use of microbial cleaning products increases substantially, among others. Added to this complexity is the fact that advancements in scientific knowledge and for that matter microbial genetics have changed microbial phylogeny and taxonomy considerably over the years (OECD, 2003; OECD, 2015). Thus, there is need for gathering updated information towards a harmonized platform for revision of existing regulations and to form the basis for potential novel regulations as widespread application of microbial cleaning products ensues. This report explores these concerns in relation to the current situation in Norway and provides scientific knowledge and recommendations to the guidelines on the regulation of microbiological products. Notably, assessment of genetically modified organisms is excluded in the mandate of this assignment as these are regulated under other legislation and procedures.

3 Evaluation and recommendations to improve the guidelines for the regulation on microbiological products

This chapter has been jointly written together with the working group on "Health and environmental risk assessment of microorganisms used in bioremediation" and subsequently specified for microbial cleaning products. The guidelines to the regulation of 22 January 1998 no. 93 on the declaration and labeling of microbiological products consists of 9 main parts with a total of 28 specific questions, outlined accordingly as separate sections below (See Appendix I).

Although the guidelines require extensive documentation of microbial cleaning products, the Panel still considers that there is need for further information and a general modernization in the methodological approach. This is discussed in more detail below.

3.1 General information

Part 1 of the declaration (questions 1-9) and the guidelines is generally satisfactory in the current form. However, the contact details (e.g. web address) should be updated and the data sheet following the product (or another documentation stating its trade name) should be provided.

3.2 Composition of the product

Purpose of Part 2: To identify all components of the product, both microorganisms and chemical components.

This part needs to be re-evaluated in order to provide more accurate requirements for specification of the microbial and chemical composition of the product.

The Panel recommends that the guidelines are revised and rewritten in the direction of the demands placed in the Nordic Ecolabel licence (Nordisk Miljømärkning/Swan label). For a cleaning product to be awarded a Nordic Ecolabel licence, a series of requirements must be fulfilled. These include not only that the applicant provides necessary information, but does also list several properties that either are prohibited or required. The requested properties need to be documented by laboratory tests.

Only cleaning products containing microorganism intended for the professional market are allowed under the label. Among these, only products which are used to clean fixed surfaces

(floors, walls etc.), while spray products or products marketed to be used with a spray application containing microorganisms not are allowed labelling.

The Swan label does not allow any products that contain substances classified according to the CLP Regulation (EC) No 1272/2008 with amendments (among them commission regulation (EU) No 286/2011 of 10 March 2011). With "substances" the regulation means "a chemical element and its compounds in the natural state or obtained by any manufacturing process". The regulation includes substances classified as respiratory or skin sensitizing in Category 1, 1A or 1B (unclassified, strong and other sensitizers, respectively) marked with Hazard Statement H334 (May cause allergy or asthma symptoms or breathing difficulties if inhaled), Category 1, 1A or 1B with Hazard Statement H317 (May cause an allergic skin reaction) or with following warning included on the package: "Contains (name of sensitising substance). May cause an allergic reaction." The categorization of substances is described in CLP Regulation (EC) No 1272/2008 Annex 1, point 3.4. "Respiratory or skin sensitisation" with amendments in commission regulation (EU) No 286/2011, Annex 1, point 3.4.2.1 for respiratory sensitizers and point 3.4.2.2 for skin sensitizers. The substances' classifications as respiratory or skin sensitizers are based on clinical evidence of sensitization in humans and/or animal experiments. Biological material that may act as sensitizers is not regulated in the legislation referred to.

Taken together, the Swan label has more accurate requirements for specification of the microbial content (Question 10 & 11), and with some elements (e.g. more modern genetic methods) that represent an improvement compared to the Norwegian regulation. The requirements for the specification of chemical content (question 12) is considerably more elaborated, is more accurate, but may address issues as: prohibition against certain types of preservatives, colouring agents, perfumes, etc., see e.g. §K6, pp. 25-31, regulates water- and energy consumption (p. 7), and types of packaging (p. 53), that may lie outside the scope of this review.

The Swan label specifically addresses ecotoxicity (K10, pp. 37-39) and health issues for user and exposed persons (K3, K4, K5, K6, K7; K8 & K9, pp 19-37).

Question 10. Specify which microorganisms are present in the product

The taxonomic affiliation of the organisms present in the product needs to be specified to at least species, preferably strain level. The identification could be e.g. 16 S ribosomal DNA or similar specific methods. A list of suitable methods is given in table 1, (p. 927) in (Emerson et al., 2008).

As pointed out by Thomas and Versteeg (2013) in the proceedings from the International Workshop to address Risk Assessment and Risk Management Challenges and Opportunities Relating to Microbial-Based Cleaning Products held in Canada 2013, an accurate identification of the microorganisms present in the product is crucial for a proper risk assessment. Allowing a specification to genus-level (or even unspecified consortia), will likely both increase the risk for allowing the incorporation of unknown pathogenic microorganisms

in the product, and may increase the risk for eventual horizontal transfer of antibiotic resistance genes from other resistant microorganisms.

Concern for antibiotic (AB) resistance: Rather than specifying that the microorganism shall not be resistant to a specific list of antibiotics, we recommend that the regulation specifies the classes of antibiotics to which the organisms shall not be resistant to. AB resistance is partly due to mutations, and partly due to horizontal gene transfer. Some of the resistance mechanisms are generic and highly transferable, and focus on the classes of AB will address this issue better.

This area also would benefit from the new molecular genetical methods, where genes coding for AB resistance both to AB classes and specific ABs can be identified (McArthur et al., 2013).

An overarching improvement in quality assurance of the data would be to specify that the laboratories providing the necessary data, are accredited to the relevant ISO-standard.

The state (living, dead, inactivated) of the microorganism present should be specified, and if killed or inactivated: the method for inactivation should be specified.

Question 11. Specify the concentration(s) of the microorganism(s) present in the product itself.

The concentration of the microorganism(s) in the undiluted product and the anticipated end-user dilution must be provided. CFU enumeration is one option, but may miss strains growing poorly on standard agar counting plates. Currently, more powerful techniques are developed for a number of applications. Some of these allows specification of metabolically active vs passive cells. "Best practice" for documentation of concentration must be used, and documentation of the choice of method must be given.

Question 12. Specify the chemical substances present: CAS no., chemical name, EC number, classification, content as percentage by weight.

The Panel consider that these issues are fairly well covered by existing regulations. However, if the more stringent approaches are called for, useful approaches can be found in the Swan label. We have no further comments to this question.

3.3 Information on any pathogenic properties of the microorganisms

Purpose of Part 3: to obtain information on any pathogenic properties of the microorganism(s)

Part three of the declaration should be extended in order to give stronger emphasis also to pathogens of animal and plants, as this is significant in environmental risk assessment.

Generally, the regulation on declaration and labelling of microbiological products, the declaration form and "Guidelines for completing the declaration required according to the regulations relating to the declaration and labelling of microbiological products for applications that may involve their release to the outdoor environment" focuses on human pathogens without giving adequate emphasis to pathogens of animals and plants.

General comments:

- Again there seem to be a general lack of accuracy when it comes to specification of what microorganisms that are included in the product. Without proper taxonomic classification, no meaningful risk assessment is feasible, and the applicant should be obliged to name the species and strain(s) in question as specific and accurate as possible.
- There seem to be a lack of emphasis on environmental side effects, especially on
    - the potential for persistence and spread in the environment
    - the potential for pathogenic effects on different wild species (vertebrates, arthropods, plants)
    - the potential for pathogenic effects on agricultural plants
    - the marine environment (which should be very important in Norway)
- There is little emphasis on the effects with increased use and accumulation, persistence and spread, both indoor, in terrestrial and aquatic environments. The questionnaire should among other things ask about the survival ability of the microorganisms in question in different types of environment.
- There seem to be a lack of thinking about the potential for disturbance of the ecological environment and resulting long-term effects on microbial community.

Question 13. Has it been reported that the microorganism(s) or other strains of the same species have caused disease/injury in humans, animals or plants (name of the disease, host organism, disease mechanism)

Instead of relying on its own, enclosed list of pathogenic organism, the classification of the microorganisms in question should be based on well-known public lists (i.e. EU directive 93/88/EEC, October 1993; NIH Guidelines on Recombinant DNA (April 2002); Canadian Laboratory Biosafety Guidelines (2nd ed. 1996); CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (4th Edition 1999) or Forskrift om tiltaksverdier og grenseverdier for fysiske og kjemiske faktorer i arbeidsmiljøet samt smitterisikogrupper for biologiske faktorer (forskrift om tiltaks- og grenseverdier); Vedlegg 2: Liste over klassifiserte biologiske faktorer (smitterisikogrupper) https://lovdata.no/dokument/SF/forskrift/2011-12-06-1358#KAPITTEL_9.) However, none of these classifications are based on the environmental risk of the microorganisms they deal with, and the reference should preferably be one that in addition to assessing risk for human health, also take into account the potential hazard for the environment.

If a microorganism is not listed in any of the recommended sources, or as an alternative to relying on such classification lists, other reliable sources may be used. This may be based on a literature review of the peer-reviewed scientific literature. If there is any uncertainty

concerning the risk group the higher group is to be chosen until it has been made clear that the risk justifies placement in a lower risk group.

Not only the pathogenic properties of the actual strain(s) and different strains of the same species should be declared, but also species in the same genus and closely related genera, should be evaluated.

If the microorganism is related to any pathogens, it should be declared how the organism in the product/product organism differs from any closely related disease causing strains with respect to safety. What changes have been made to the organism (e.g. attenuation) to expect that it will not cause similar diseases like the related strains. Or how the product organism has been characterized to ensure it is not a pathogenic strain.

Even for non-pathogens, their ability as opportunistic organisms should be described, e.g. against immunocompromised individuals; this can be derived from the published literature. Concerning this, if the organism has been shown to cause diseases in immunocompromised individuals, a survey of the immune status of individuals in and around the environment of application should be conducted to help devise means of mitigating the risk of their infection.

Knowledge about genetic exchange between the strain in question and other strains and species should be asked for (see point 21).

It should be declared where and in which amounts the microorganism(s) normally are found. If it is ubiquitous in the environment, this may indicate lower risk of disease and other adverse effects in the environment (see section 4.6).

The declaration should include documentation of knowledge (obtained from declarant's own research or published literature) on

- acute infectious disease in humans
- effects of chronic exposure in humans (allergies, sensitization etc.)
- infectious disease in terrestrial animals
- infectious disease in aquatic (including marine) animals
- effect on insects and other arthropods, especially pollinating insects
- pathogenic effects on plants
- effects on microbiological ecology in environments during long-term use and/or use of large amounts
- persistence in the environment, accumulation, both indoor, soil and aquatic

The declaration should also state to which degree there is sufficient knowledge on occurrence of virulence and resistance factors of the strain that may

- be exchanged to pathogenic microorganisms
- cause the current strain to act as an opportunistic pathogen

It should be stated if there is any knowledge on the interaction between other ingredients of the cleaner and the microorganism in this context.

The agent should be grouped in a Risk Group (1-4) and the underlying Biosafety Level Definition has to be stated, according to e.g. European Economic Community (DIRECTIVE 93/88/EEC, Oct. 1993), NIH Guidelines on Recombinant DNA (April 2002), Canadian Laboratory Biosafety Guidelines (2nd ed. 1996) or CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (4th Edition 1999) (see Appendix II).

Risk Group Databases can be found here:

1. Forskrift om tiltaksverdier og grenseverdier for fysiske og kjemiske faktorer i arbeidsmiljøet samt smitterisikogrupper for biologiske faktorer (forskrift om tiltaks- og grenseverdier); Vedlegg 2: Liste over klassifiserte biologiske faktorer (smitterisikogrupper) https://lovdata.no/dokument/SF/forskrift/2011-12-06-1358#KAPITTEL_9

2. American Biological Safety Association https://my.absa.org/tiki-index.php?page=Riskgroups

3. Public Health Agency of Canada http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php

If a microorganism is not listed in any of the recommended sources, other reliable sources may be used. There may be a publication in the peer-reviewed scientific literature that describes the agent. If there is any uncertainty concerning the risk group the higher group is to be chosen until it has been made clear that the risk justifies placement in a lower risk group.

Concerning the Swan Label, only microorganisms belonging to "Risk group 1" (unlikely to cause human disease) according to Directive 2000/54/CE are allowed. It specifies that a cleaning product is not eligible for labelling if it contains *E. coli, Streptococcus* spp., *Staphylococcus aureus, Bacillus cereus* or *Salmonella* spp. upon analyses with specified test methods or equivalent methods, and the applicant has to document this.

The potential role of the microorganism in the product acting as allergens, has been examined through a number of searches. While the immunological tolerance to oral ingestion of food (and concomitant bacteria) in general is high (Chinthrajah et al., 2016), "roles for the commensal microbiome in promoting oral tolerance and the association of intestinal dysbiosis with food allergy are discussed. Growing evidence supports cutaneous sensitization to food antigens as one possible mechanism leading to the failure to develop or loss of oral tolerance" (Chinthrajah et al., 2016), op. cit.), (Piras et al., 2016).

In addition, the relation between the microbiome of the respiratory tract and asthma has gained more focus recently, see e.g. Earl et al. (2015) and Huang and Boushey (2015).

Hence, the immunological implications (including cross allergenicity) of dermal or respiratory exposure for microorganisms in microbial cleaning products need to be further explored.

Question 14. Specify the tests that have been made to ensure that the product is not contaminated with unwanted microorganisms, particularly pathogenic microorganisms.

Again, here the Panel recommends a revision of the guidelines, especially with emphasis on the methodological approach.

In the guidelines it is stated that "For products consisting solely of identified microorganisms, it will be sufficient to test for general contamination". 'General contamination' should be clearly defined. Is there contamination by human, animal and plant pathogens? A molecular approach should be combined with the culture method given that culture method may be unable to detect pathogenic bacteria forms which when they get into the body can proliferative and cause diseases. This is especially so for viruses which can only be cultured in tissue culture in adequate laboratory facilities. A suggestion is to use high-sensitivity molecular detection to first screen for presence of pathogens, potential pathogens, toxin-producing microorganisms and AB resistance genes. Pathogens which cannot be easily detected by standard culture methods will therefore be considered further. Also, this approach first provides an overview of the pathogens/potential pathogens present, which will then guide the culture approach and makes the culture approach more targeted.

The declaration should provide information about

- how the quality control and assessment is performed.
- which procedures that are in place for securing absence of pathogens
- any testing for known relevant virulence or resistance factors that may increase health or environmental risk
- how the microbial content within the products develops with time through and after shelf life. May contamination or other changes occurring during use increase virulence?

The application in our opinion should not necessarily rely on specific methods, as long as the methods described are scientifically adequate. A multiphasic approach should be emphasized.

The product needs to be pure before being dispersed to the environment. A pure product will ensure adequate characterization of the microorganisms and the eventual breakdown product during processing (i.e. before release to the environment).

Microorganisms must not contain any of the following pathogenic species: *E. coli*, *Streptococcus* (*Enterococcus*) spp., *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella* spp.

Question 15. Specify recommended precautions to be taken in connection with use of the product (respiratory equipment, personal protective equipment, hygienic measures, etc)

This section need to be elaborated. In our opinion a declaration should include information on:

- the intended use and instructions for use.
- the user groups, for example if the product is suitable for immunocompromised persons, elderly, young, infants, pregnant etc.
- if the product can be used in environments with food production or animal production?
- specific precautions need to be taken with waste, containers etc.
- specific precautions for use and personal protection
- storage in relation to how storage influences the microbiological composition and pathogenic properties

3.4 Information on inactivation of microorganisms

Purpose of Part 4: to determine whether or not the product contains live microorganisms. Unless the presence of live microorganisms is important for the performance of the product, they should be inactivated.

The Panel considers the guidelines to part 4 to be generally satisfactory in the current form.

Question 16. Does the product contain live microorganisms, including viable spores (bacteria and fungi), or cysts (protozoans)?

The Panel recommend that the exposure time is also specified in the declaration.

3.5 Information on where and how the product is to be used

Purpose of Part 5: to obtain information that gives an indication of the risk of unwanted establishment and dispersion of the microorganism in the environment in which the product is intended for use.

The Panel recommends that the guidelines are extended for part 5. The Panel especially suggests more emphasis concerning possible hazards for particular risk groups and clarification on where and how the products should be used or should not be used. Possible short or long term impacts for the environment, and methods for inactivation or for sanitary quarantine of the contaminated area when used unintentionally should be discussed.

Question 17. Where is the product intended to be used, and is this environment appreciably different from the environment from which the microorganisms have been isolated?

Since the main focus of part 5 is to obtain information on unintended effects, it would be necessary to state likely environments (different from the environment of exposure) which can also inadvertently be exposed to the product and in which the microorganisms can thrive. It will be necessary to describe similarities between the intended and these likely-to-

be exposed unintended environments; measures on how the product(s) will be excluded from these environments should be clearly stated.

In case of spill to an unintended environment, methods for inactivation or for sanitary quarantine of the contaminated area should be provided.

The risks linked to the use of strains that belong to species known to include opportunistic pathogens and possible hazards for particular risk groups (YOPI – young, old, pregnant, immune compromised) should be clarified; this is linked to possible restrictions in, e.g. hospitals, retirement homes, and child care.

In the Netherlands, the Dutch Food and Consumer Product Safety Authority (VWA) recommends not to use microbial cleaner in areas of food processing and preparation and also not with particular risk groups (YOPI). More recently, they also advised against the use in hospitals based on the same reasons.

The Panel recommends clarification on where and how the product should be used/not be used, e.g. whether the products can be used on surfaces in contact with food and if the products can be used with spray application. If so, a justification for these applications should be provided.

Question 18. How are the microorganisms released to the environment?

Even if declarants provide information supporting the exposure assessment for the microorganism, models for predicting environmental fate or expression are lacking.

It should be discussed what kinds of shift in the microbial community and local community can be expected in the receiving environment, especially if exposure is chronic and frequent.

It seems it has been taken for granted that the form of the organism to be released (spores/endospores/cysts/live vegetative cells) will also inform the type of method to be used in the release (spray, liquid or solid product types). Given that companies provide only requested information ignoring information not explicitly requested, it might be necessary to also state the form of the organism to be released and the method of spread that is adopted.

Question 19. With respect to the microorganisms, what are the typical concentrations, quantities (quantity per unit of volume, weight or area) and frequencies of application, and the total number of applications?

Data on the persistence of vegetative forms and spores in the environment should be provided, and conditions for germination of the latter should be described.

Prescriptions related to further application of the product need to be provided. Particular attention should be given in case the microorganism establishes itself in the environment (sporulation).

Information on the colonization ability (including competitive ability in relation to closely related species) in the new environment should be provided.

Some of the above-mentioned recommendations are adequately addressed by the Swan label, and could consequently be consulted in a revision of the guidelines.

3.6 Description of the microorganism

Purpose of Part 6: to obtain information on any traits of the microorganisms that have a strong bearing on the risk of injury to health or environmental damage.

The Panel suggest that this part is revised. Some specific considerations are discussed below.

"Environmental Damage" is not sufficiently/explicitly defined. The document focuses only on the introduction of foreign gene into the ecosystem. The environment can also be permanently altered (or damaged) if the introduction of the new organisms results in the extinction of the naturally existing closely related species (see also comment on Question 19).

Question 20. Have the microorganisms been deliberately altered since their isolation, and if so how?

The effect of the alteration on the genetic makeup and physiology of the organisms should also be determined; e.g. a comparison with the isogenic parental wild type at genomic and physiological (proteomic and metabolomics levels).

Although this document emphasizes deliberate alteration, non-deliberate (unexpected alteration) should also be determined, for example, after the isolate would have been passaged several times. In such cases the strains current environment (laboratory or Culture center) will be quite different from the original environment of isolation. Information such as passage number and duration of storage in the Culture Collection Centre will be useful. Therefore, information on how the Master Seed Culture and Working Cultures are maintained is necessary.

It is also necessary to state what measures will be taken in the case of a laboratory adapted strain or strain obtained from a Culture Collection Center that has been in the 'cold' for ages or passaged multiple times. In such cases the strains current environment (laboratory or Culture center) will be quite different from the original environment of isolation. Information such as passage number, duration of storage in the Culture Collection Centre will be useful.

Question 21. For products containing bacteria: what pattern of resistance do they show to antibiotics (including synthetic antibacterial agents)?

Rather than specifying a list of specific antibiotics employ generic classes of antibodies as with the Swan label. The latter seems more adaptable to new emergent antibodies and are well suited to (if necessary) rank the risk for antibiotic resistance based on resistance patterns / mechanisms (some of which may be transferable). For detail, the Swan labelling specify that the microorganisms cannot show antibiotic resistance to aminoglycosides, macrolides, Beta-lactam, tetracyclines, fluoroquinolones or other quinolones according to EUCAST or Nordic AST or other equivalent method. This is a disk diffusion method (Nordic AST refers to EUCAST). For comparison, the Norwegian legislation asks for resistance against amoxicillin/clavulanic acid, ampicillin, cephalothin, chloramphenicol, erythromycin, fucidic acid, lincomycin, methicillin, norfloxacin, oxytetracycline, penicillin, trimethoprime/sulfamethoxazole and vancomycin.

Question 22. Do the microorganisms have special survival mechanisms, for example the formation of spores in bacteria?

Some microorganisms form survival and dispersal structures. These are called endospores in bacteria, spores and conidia in fungi and cysts in protozoans (though the term spores is often used for protozoans as well). Adverse environmental conditions often trigger the formation of spores and spore like structures.

3.7 Ecological effects related to degradation processes

Purpose of Part 7: to obtain information on any environmental effects of the product, with special emphasis on the formation of harmful intermediate products during the degradation process.

It is specified in the guidelines that it is only necessary to answer questions 23 and 24 if the microbiological product is intended for use in the degradation of pollutants. These questions will consequently not be considered for microbiological cleaning products.

Question 25. Can the product have undesirable effects on important natural microbial processes in the environment, for example nitrogen/phosphorus cycles and carbon mineralization, or by altering pH or oxygen concentration?

The purported working mechanisms for the efficacy of the microbial cleaning products are through several of these metabolic pathways. However, as the microbial cleaning products are mainly for indoor use and the total microbial load *in situ* is relatively limited, the microbial flora at the release site can be expected to be adapted to the local environment. Hence, we consider harmful effects would require either a massive release event, or that the general consumption (and concomitant chronic release) of such products increases considerably above the current use. We do, however, consider that these questions merit further investigation.

3.8 Other relevant information

Question 26. Give any other information in the form of empirical or test data that is relevant to the hazard to health or the environment posed by use of the microbiological product, and to which the importer/manufacturer has or should have access.

We consider that these issues are fairly well covered by existing regulations. We have no further comments to this question.

3.9 Overall assessment of risk to human health and the environment

Question 27 Give an overall assessment of the risk to human health and the environment posed by use of the product.

The guidelines here are very open and general. Specific guidelines should be provided on how this should be done, as for instance demand simple risk assessment diagrams.
The question should be more specified. A risk assessment should be given for:

1) Health hazards caused by microorganisms in the product
   a) Intended use of the product
   b) Wrong (unintended) use of the product

2) Environmental hazards caused by microorganisms in the product
   a) Intended use of the product
   b) Wrong (unintended) use of the product

3) Health hazards caused by chemicals in the product
   a) Intended use of the product
   b) Wrong (unintended) use of the product

4) Environmental hazards caused by chemicals in the product
   a) Intended use of the product
   b) Wrong (unintended) use of the product

4 Potential health and environmental implications of the microorganisms

A reliable taxonomic designation allows for the appropriate assessment of a microorganism's infectivity, virulence and overall pathogenicity. Thus, to achieve holistic risk assessment of a given microorganism, taxonomic designation is the fundamental determinant of its potential hazard to human health and the environment (OECD, 2015). This includes but is not limited to its ability to produce toxic metabolites, allergens and potential effects on vulnerable populations (YOPI) (OECD, 2015; Spök and Klade, 2009).

With regards to the potential health and environmental impacts of the microorganisms employed in cleaning products, we consider the major challenges to be proper taxonomic identification, exposure scenarios, effects in the recipient ecosystem, and exposure risks for vulnerable groups in the population. Taxonomic identification is unclear and poorly specified. In order to give a proper risk assessment, the accurate identity of the bacterial strains used should be known (OECD, 2015; Spök and Klade, 2009). With current molecular methodologies, not only conserved species-identifying DNA is in principle accessible for analysis (to e.g. strain level) for well-known species , but it is also possible to detect the organisms' (own or acquired) nucleic acids coding for unwanted traits (toxins, pathogenicity, etc.). While the technical tools are available, the sequence libraries facilitating an accurate identification for poorly described / undescribed microorganisms remains incomplete. Notably, considerable uncertainties as well as knowledge gaps exist for reliable or definite conclusions to be drawn regarding hazard and exposure assessments of microbiological cleaning products (see section 6 & 8).

Although microbiological cleaning products may present fewer health hazards and are considered environmentally friendly or less bio-persistent, very few quantitative assessments of these products employed in green cleaning technologies exist. Specifically, there are limited data evaluating respiratory, dermal, or other hazards associated with specific green cleaning products (Quinn et al., 2015). In addition, potentially harmful cleaning exposures are not only a function of the product characteristics, but also a function of the mode of application (spraying, wiping etc.) and the work practices and conditions with which they are used (Quinn et al., 2015).

Moreover, there is limited data on end-user exposure to micro-organisms and spores during the routine application of their products. As of 2013, no such data had been made available to regulators and external experts (Thomas and Versteeg, 2013). Furthermore, the way in which microbial cleaning products are used presents particular challenges for exposure assessment. A product requirement for repeated application and the fact that micro-organisms are intended to stay on surfaces, undergo sporulation and germinate again create conditions for chronic exposure. In many cases, these products are sprayed onto hard surfaces, mattresses, carpets and upholstery, which can lead to chronic respiratory exposure

(OECD, 2015). These considerations are relevant for risk assessment, because in the scientific literature some microorganisms are considered to be respiratory sensitizers. *Bacillus spp*. used as pesticides, for instance, are generally considered by regulators to be respiratory sensitizers (OECD, 2015). Risks resulting from chronic exposure in general and to vulnerable groups are not sufficiently clear. The study conducted by Spök and Klade (2009) reported that a significant number of products on the market harboured microbial contaminants some of which were known to be food contaminants and opportunistic pathogens. As a result, the use of these products were prohibited in certain settings such as healthcare institutions, food facilities and areas with vulnerable people, namely the immuno-compromised, infants, the elderly, and pregnant women.

As discussed in other sections, environmental impacts are not expected as a direct result of the use of microbial cleaning products presently. However, issues may arise should these products be manufactured, imported and/or used in exponentially greater quantities in the future. This may result in significant environmental releases that will warrant greater scrutiny from a regulatory perspective eventually. Additionally, the safety reassurances provided by producers of microbial cleaning products do not cover properties related to allergenicity, sensitization, plant pathogenicity and environmental impacts as discussed in the introductory sections.

Notably, the need for sustainable and effective alternatives to routine cleaning and disinfection is understood, given the recent and fast evolution of multi-resistant pathogens in healthcare facilities (Vandini et al., 2014). A recent study demonstrated that microbial (probiotic-based) cleaning is more effective in the long-term lowering of the number of healthcare associated infection-related microorganisms on surfaces, when compared to conventional cleaning products, even those containing disinfectant molecules such as chlorine. The first indications on the percentages of healthcare associated infections in the trial hospitals monitored on a continuous basis throughout the study are very promising and may pave the way for a novel and cost-effective strategy to counteract or (bio)control healthcare-associated pathogens (Vandini et al., 2014). When it comes down to risk management, more research is needed on the effectiveness and health implications of green cleaning in all types of settings.

5 Uncertainties

For the purpose of clarity and transparency in risk assessment processes, it is recommended that assessments identify sources of uncertainties and state exquisitely their subsequent impact on the overall assessment outcome since this is critical in the subsequent selection of risk management options (EFSA draft opinion).

In our view, the degree of uncertainty regarding the effects of increased use of microbial cleaning products might have on health and environment, inherently has to be high. This uncertainty is partly related to the dearth of scientific research on adverse effects of the use and release of microbial cleaners into the Norwegian environment. Even if these knowledge gaps (see section 8) were filled before an eventual release of a certain microbial cleaner into a complex ecosystem (including the wide variety of human behaviour into the concept of "ecosystem"), uncertainty of potential impacts on health and environment may still prevail.

A major difference between traditional chemical cleaning products and their microbial alternatives when it comes to risk assessment, may be said to be the higher degree of complexity and hence unpredictability of the microorganisms interaction with the environment. It is, for example, unattainable to reach scientific consensus and "final conclusions" on the following questions:

- What is the risk of evolution of pathogenicity in a certain strain of microbes that is continuously exposed to our indoor environment over a long period of time?
- What is the risk of recombination with other naturally occurring or artificially introduced microbes that may lead to achievement of or creation of new virulence factors over a long period of time?
- What is the impact on the human immune system, for example in term of hypersensitivity disorders, of continuous exposure of the microbes and their products?
- When released into the environment, to which degree may microorganisms from cleaners change the microbial ecology of various ecosystems?
- When released into the environment, to which degree may they act as pathogens to, or disturb the natural microbial flora of, higher organisms not commonly exposed to such microbes?

5.1 Summary of uncertainties

Given the unfathomable complexity of the interactions between even a well-described microorganism and the biotic and abiotic environment they are released into, and the

inherent lability of biological systems, there will always be some uncertainty about the long-term impact on health and environment of the use of microbial cleaners.

Given that we deal with live organisms, we have to expect the unexpected. However, this is also true if we reject the introduction and increased use of microbial cleaners and continue to use chemical cleaners, disinfectants and antibiotics as today.

Consequently, decisions will have to be made before conclusive scientific evidence is available. The resulting ambiguity of a mixture of scientific knowledge and non-objective assumptions may, however, be acceptable to the public if the processes and decisions are translucent and the uncertainty well communicated (Van Der Sluijs, 2005).

6 Conclusions (with answers to the terms of reference)

Based on scientific assessment of the information requirements laid down in the declaration form of the Regulation on microbial products, VKM concluded that the information requirements in their current form are not sufficient to conduct a health and environmental risk assessment of the use of microbial cleaning products in Norway.

Consequently, VKM has suggested updated information requirements that producers and importers must / should meet that can be used as a basis for the risk assessment of health and environmental risks associated with microbial cleaning products in Norway.

The following include our main concerns:

- Accurate taxonomic identification is a key step in risk assessment. There seems to be a general lack of accuracy when it comes to specification of the microbial content and concentrations (metabolically active vs. inactivated or dead cells) that are included in products. The reliability of this step remains uncertain if the producers are allowed to provide information with poor taxonomic resolution. Such practice makes it difficult, if not impossible for risk assessors to provide health and environmental risk assessments on these microbial cleaning products.
- The application in our opinion should not necessarily rely on specific methods, as long as the methods described are scientifically adequate.
- There seems to be lack of emphasis on environmental impacts, especially on the potential for persistence and spread in the environment (terrestrial or aquatic), the potential for pathogenic effects on domestic or wild vertebrates, arthropods or plants.
- The state (living, dead, inactivated) and form (vegetative, viable spores (bacteria and fungi) or cysts (protozoans)) of the microorganism present should be specified.
- The declaration should provide information about the procedures and quality controls securing a product without contaminations, pathogens, or known relevant virulence or resistance factors that may increase health or environmental risks.
- In our opinion, a declaration should include information about intended use and instructions for use, if specific precautions (personal protection, waste, containers etc.) need to be taken.

The proposed information requirements can be obtained using internationally recognized methods available today. Wherever relevant, the criteria laid down in the Nordic Swan Ecolabel have been referred to. Critical knowledge gaps for the risk assessment of microorganisms have been included.

Assessment of genetically modified microorganisms (GMO) is not included as these are regulated under other legislation and procedures.

7 Data gaps

For the purpose of this report, specific products on the Norwegian market and the extent of usage were not included. Data gaps identified in hazard assessment, exposure assessment and efficacy are outlined below:

- Knowledge on the risks related to chronic/long term exposure to dusts and aerosols is limited.
- Knowledge on the risks linked to the use of strains which belong to species known to include opportunistic pathogens and possible hazards for particular risk groups like pregnant women, children, elderly, immunocompromised persons, people with respiratory ailments, etc. (OECD, 2015; Spök and Klade, 2009).
- There is limited information on the shelf life or viability of the micro-organisms contained in microbiological cleaning products. However, a decline in the number of micro-organisms over time is suggested (Spök and Klade, 2009).
- *The effectiveness compared to chemically based cleaning products* (OECD, 2015). In the absence of common standardized methods that are appropriate for testing all-purpose cleaners, not to mention the general lack of tailored/specific methods applicable to microbial cleaners, third-party verification of the efficacy claims made by manufacturers is almost impossible and thus scarce. The same is true regarding detailed information on the mode of action of these micro-organisms in cleaning products.
- *Environmental impacts*: While the volumes released under current domestic or commercial use is limited, environmental issues may arise, should microbial-based cleaning products be manufactured, imported and/or used in greater quantities than what is currently known. These could result in significant environmental releases that may warrant greater scrutiny from a regulatory oversight perspective (OECD, 2015; Spök and Klade, 2009).
- *The available information on the various mechanisms of actions of the microbes is considered insufficient.* Partly as a consequence of lack of transparency, but also due to lack of (detailed) knowledge of some products (Spök and Klade, 2009).

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9 Appendix I

Regulation of 22 January 1998 no. 93 on the declaration and labelling of microbiological products and its guidelines

https://www.regjeringen.no/en/dokumenter/declaration-and-labelling-of-microbiolog/id440456/

10 Appendix II

European Economic Community (DIRECTIVE 93/88/EEC, Oct. 1993), NIH Guidelines on Recombinant DNA (April 2002), Canadian Laboratory Biosafety Guidelines (2nd ed. 1996) and CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (4th Edition 1999)

European Economic Community (DIRECTIVE 93/88/EEC, Oct. 1993)

(1) Group 1: biological agent means one that is unlikely to cause human disease;

(2) Group 2: biological agent means one that can cause human disease and might be a hazard to workers; it is unlikely to spread to the community; there is usually effective prophylaxis or treatment available;

(3) Group 3: biological agent means one that can cause severe human disease and present a serious hazard to workers; it may present a risk of spreading to the community, but there is usually effective prophylaxis or treatment available;

(4) Group 4: biological agent means one that causes severe human disease and is a serious hazard to workers; it may present a high risk of spreading to the community; there is usually no effective prophylaxis or treatment available.

NIH Guidelines on Recombinant DNA (April 2002)

(1) Risk Group 1 (RG1): agents are not associated with disease in healthy adult humans.

(2) Risk Group 2 (RG2): agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.

(3) Risk Group 3 (RG3): agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.

(4) Risk Group 4 (RG4): agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.

Canadian Laboratory Biosafety Guidelines (2nd ed. 1996)

(1) Risk Group 1 (low individual and community risk): This group includes those microorganisms, bacteria, fungi, viruses and parasites, which are unlikely to cause disease in healthy workers or animals

(2) Risk Group 2 (moderate individual risk, limited community risk): A pathogen that can cause human or animal disease but under normal circumstances, is unlikely to be a serious hazard to healthy laboratory workers, the community, livestock, or the environment. Laboratory exposures rarely cause infection leading to serious disease; effective treatment and preventive measures are available and the risk of spread is limited.

(3) Risk Group 3 (high individual risk, low community risk): A pathogen that usually causes serious human or animal disease, or which can result in serious economic consequences but does not ordinarily spread by casual contact from one individual to another, or that can be treated by antimicrobial or anti-parasitic agents.

(4) Risk Group 4 (high individual risk, high community risk): A pathogen that usually produces very serious human animal disease, often untreatable, and may be readily transmitted from one individual to another, or from animal to human or vice-versa directly or indirectly, or casual contact.

CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (4th Edition 1999)

(1) BIOSAFETY 1 is suitable for work involving well-characterized agents not known to cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment.

(2) BIOSAFETY LEVEL 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment.

(3) BIOSAFETY LEVEL 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route.

(4) BIOSAFETY LEVEL 4 is required for work with dangerous and exotic agents which pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease.