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# Assessment of risks related to agricultural use of sewage sludge, pig and cattle slurry

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# SAMMENFATNING

Det Økologiske Erhvervsteam, nedsat af Miljø- og fødevareministeren, anbefalede i April 2017 under overskriften 'Økologien som eksperimentarium for udvikling af den cirkulære bioøkonomi' at økologerne skal have mulighed for at anvende næringsstoffer fra behandlet husspildevand.

Forudsætningen for fremtidig anvendelse af næringsstoffer fra behandlet spildevand i økologisk jordbrug er at kvalitetskriterier kan overholdes, forklares til og forstås af forbrugere. En nødvendig afklaring i forhold til en evt fremtidig anvendelse er udarbejdelsen af denne rapport, som giver et overblik over risikofaktorer for mennesker og jordmiljøet ved gødskning med kvæg og svinegylle, samt biogødning fra spildevand. Erhvervsteamet forudså at Danmark, afhængigt af rapportens udkomme, kunne vælge at arbejde for at EU's økologiregler udvides, og muliggøre recirkulering af næringsstoffer fra behandlet spildevand og andre mulige acceptable afledte produkter.

Formålet med nærværende rapport var derfor at skabe et overblik over de humane såvel som miljømæssige risici, der er forbundet ved brug af henholdsvis svine- og kvæggylle og spildevandsslam som gødning på landbrugsjorde. Rapporten bygger udelukkende på den tilgængelige litteratur, og har søgt viden om danske forhold. I de tilfælde hvor der ikke fandtes relevant dansk viden, er der opsøgt viden fra tilsvarende lande.

Følgende stofgrupper indgik i vurderingen:

Antibiotika resistensgener, metaller, chlorophenyler, dioxiner, furaner, halogenerede alifatiske hydrocarboner (HAH), lineære alkylbenzenesulfonater (LAS), polyaromatiske hydrocarboner (PAH), polybromerede diphenyl æthre (PBDE), polychlorerede biphenyler (PCB), poly- og perfluorinerede alkylerede substanser (PFAS) phenoler, phosphat-triestre, phtalater, polychlorinerede naphtalener (PCN), polychorerede alkaner (PCA), triclosan, triclocarban, veterinær- og humane medicinrester, og østrogener

**RISIKO FOR HUMAN SUNDHED**

En kvalitativ gennemgang baseret på 'state of the art' litteratur blev foretaget for potentielt skadelige tungmetaller, medicinrester og spredningen af antibiotikaresistens. Disse elementer er på nuværende tidspunkt vurderet til at udgøre den største bekymring for human sundhed i forbindelse med landbrugsmæssig brug af svine- og kvæggylle samt splidevandsslam.

**Potentielt skadelige metaller**

Potentielt toksiske elementer (PTE) udgør en bekymring idet de kan akkumuleres i jorden. Metallerne fjernes udelukkende fra jorden via udvaskning og planteoptag. Mennesker kan udsættes for PTE via direkte indtag af plantemateriale. Cadmium og bly (Cd og Pb) er de mest fremtrædende af disse i dansk sammenhæng, når det kommer til direkte effekter på menneskers sundhed. En række af faktorer påvirker den biologiske aktivitet af PTE, den vigtigste af disse er pH. Ved at kontrollere pH gennem kalkning kan planternes optag af kationiske PTE (herunder Cd) minimeres. I en norsk risikovurdering af spildevandsslam blev det vurderet, indtaget af råvarer produceret på jorde beriget med slam gennem 100 år, kun ville øge Cd optaget per borger med mindre end 5% sammenlignet med baseline. Yderligere ser det ud til at niveauerne af Cd og Pb i afgrøder faktisk er faldende, på grund af de meget lavere forbrændingsrelaterede atmosfæriske udledninger i løbet af de seneste årtier.

Der vurderes at der er ringe risiko forbundet med tungmetaller ved humant indtag af afgrøder gødet med spildevandsslam.

**Medicinrester**

I godkendelsesprocessen for veterinære lægemidler bliver der ikke taget højde for den potentielle humane eksponering til veterinære lægemidler gennem gylle eller slam. Mennesker eksponeres for veterinære lægemidler gennem indtaget af animalske produkter. Dette indtag kan i nogle tilfælde være tæt på det acceptable daglige indtag (ADI), og derfor kan selv et mindre indtag via f.eks. planter (der har optaget lægemidlerne) teoretisk set resultere i en overskridelse af ADI. Der forefindes ganske få studier af transfer af veterinære lægemidler fra jord til planter, derfor bygger følgende stort set udelukkende på transfer af humane lægemidler. Et norsk studie undersøgte samtlige 1414 humane lægemidler på markedet i Norge. Af disse 1414 blev kun 14 vurderet til at overskride grænseværdier på 100 eller 10 µg/kg jord efter tilførsel af slam. De estimerede jordkoncentrationer var i alle tilfælde meget lav og lavere end den estimerede PNEC (Predicted No Effect Concentration).

Der findes ikke tilstrækkelig data til at konkludere på risikoen ved planteoptag af veterinære lægemidler fra husdyrgylle, men det anses for usandsynligt, at planteoptaget af veterinærlægemidler i gylle fra svin og kvæg er af betydning for menneskers sundhed, og det konkluderes, at veterinærlægemidler og humanmedicinske rester i spildevandsslam ikke giver anledning til bekymring.

**Spredning af antibiotikaresistens**

Baseret på gennemgangen er det den sagkyndige opfattelse, at spildevandsslam ikke udgør en højere risiko for udbredelse og overførsel af antibiotikaresistens end husdyrgødning. Anvendelse af spildevandsslam til gødningsformål skønnes at udgøre en meget lav risiko for

spredning af antibiotikaresistens i miljøet, såfremt anvendelsen sker i overensstemmelse med den danske slambekendtgørelse.

**RISIKO FOR JORDMILJØET**

Tilstedeværelsen af uønskede forbindelser i slam og husdyrgødning har meget beskedent overlap (kun 4 metaller og seks organiske forbindelser er målt i begge matricer), hvilket gør en direkte sammenligning af den kumulative risiko for animalsk gødning og spildevandsslam noget vilkårlig. Manglen på overlap skyldes sandsynligvis forskellene i oprindelsen, men også på grund af historiske forskelle i overvågningsindsatsen.

Den kumulative risikovurdering konkluderede, at der kan være en potentiel risiko ved anvendelse af slam og husdyrgødning i alle scenarier, mens svinegylle udgør en højere kronisk risikofaktor på grund af ret høje niveauer af Cu og Zn. Denne risiko vil dog mindskes når der i 2022 indføres ny regulering for medicinsk anvendelse af Zink, samtidigt med den igangværende regulering af kobber er fuldt indfaset.

Det blev konkluderet, at de undersøgte organiske kemikalier, medicinske rester og østrogener fra gylle udgør ingen eller lav risiko for jordorganismer. Det skal dog bemærkes, at viden om organiske kemikalier i dansk gylle er sparsom, og selvom gylle forventes at indeholde mindre mængder af organiske kemikalier end slam, kan gyllen indeholde stoffer, der ikke er medtaget i den foreliggende vurdering.

Evaluering af spildevandsslam som gødning viste potentiel toksicitet af phthalater og triclocarban. Konklusionen er dog ganske usikker på grund af manglen på toksicitetsoplysninger såvel som specifikke danske målinger af koncentrationer af nogle af disse forbindelser. Det anbefales derfor, at disse forbindelser undersøges, så usikkerheden kan reduceres.

Sammenfattende konkluderes det at dansk spildevandsslam ikke udgør en større risiko for human sundhed og for jordmiljøet end svinegylle.

# EXECUTIVE SUMMARY

## I Background

In April 2017, the Organic Business Development Team released a report with 25 recommendations for the Minister of Environment and Food (Det økologiske erhvervsteam 2017). Among these was a recommendation that organic farmers should have opportunities for utilizing nutrients from treated domestic wastewater for nutrient recycling.

A prerequisite for future use of nutrients from treated wastewater is, that quality requirements are met and that application can be explained to (and accepted by) consumers. In partial fulfilment of this, the business team identified a need for a scientific overview of the risks of using nutrients from treated municipal wastewater in relation to other authorized fertilizer sources – e.g. conventional animal manures. Thus, it was assumed that a comparative approach to assess potential risk of using sewage sludge and conventional manures, could usefully inform decision makers in the future regulation of organic farming systems.

Dependent on the result of the scientific investigation, the Organic Business Development Team foresaw that Denmark could chose to work to expand Annex 1 of the EU Ecology Regulation, to allow the organic farmers to use nutrients from municipal wastewater or other acceptable derived sludge products. Mobilization of support for this should be done by the Ministry of Environment and Food in collaboration with the Organic Farming Industry.

Thus, based on available literature, this report aims at creating an overview of the environmental and human risks associated with application of pig and cattle slurry as well as sewage sludge to agricultural soils. The risk evaluation was performed for the following compound groups:

- Metals
- Chlorophenyls
- Dioxins
- Furans
- Halogenated aliphatic and aromatic hydrocarbons (HAH)
- Linear alkylbenzenesulfonates (LAS)
- Polyaromatic hydrocarbons (PAH)
- Polybrominated diphenyl ethers (PBDE)
- Polychlorinated biphenyls (PCB)
- Poly- and perfluorinated alkylated substances (PFAS)
- Phenols
- Phosphate-triesters

- Phthalates
- Polychlorinated naphtalenes (PCN)
- Polychlorinated alkanes (PCA)
- Triclosan
- Triclocarban
- Medicines
- Estrogens
- Antibiotic resistance genes

Additionally the fertilizer potential of the two nutrient sources was characterized and compared.

# II Assessment of risks to human health

A qualitative assessment based on the 'state of the art' literature was made for potentially toxic elements (heavy metals), residues of veterinary and human medicine, and finally propagation of antibiotic resistance. These are currently understood to be the major concerns to human health, related to agricultural use of pig and cattle slurry as well as sewage sludge.

## II.I Potentially toxic elements (heavy metals)

Potentially toxic elements (PTEs) are of major concern since they tend to accumulate in soils through application of waste materials, but also due to atmospheric deposition. They are only removed through leaching or plant uptake – and unlike organic substances they are not degraded. Humans may be exposed to PTEs through direct ingestion of plant material and some of these elements may pose a direct threat to human health due to their toxicity (e.g. Cadmium (Cd) and Lead (Pb) ). A number of factors control the biological activity of PTEs, most importantly soil pH. By controlling soil pH through occasional liming, as is the commen practice in Denmark, the plant uptake of cationic PTEs, and most importantly Cd may be controlled. In a Norwegian risk assessment on sewage sludge it was considered that food produced from soil amended with sludge for 100 years would increase Cd intake per capita with less than 5% relative to the baseline, and this was deemed acceptable. More recently a discussion has started to evolve, based on the assertion that Cd concentrations in food produced in Europe may be generally declining. Due to much improved technologies for control of emissions related to combustion processes substantial declines of emissions of mercury (Hg), Pb, and Cd have taken place over the last 2 decades. Thus, Cd and Hg emissions have been reduced by a factor 5, while Pb emissions have reduced by a factor 15. This in turn, may have consequences for the long-term exposure of humans to Cd vis-à-vis dietary intake. According to one study, soil Cd concentrations should decrease around 14% over 100 years, at the highest allowed level of Cd in sewage sludge (100 mg Cd $kg^{-1}$ P). Therefore, it should be expected that the amount of Cd in food produced in European countries should have started to decline (albeit slowly).

## II.II Residues of veterinary and human medicine

Potential human exposure from transfer of veterinary medicinal products via manure or sewage sludge into crops is not considered in the approval process of veterinary medicine. Since the exposure from food of animal origin could be very near the acceptable daily intake (ADI), even a minor increase in the exposure from transfer from sewage sludge and animal manures to crops could in theory result in an exceedance of an ADI. As there is almost no investigation on the transfer of veterinary drugs, except antibiotics, into food the current knowledge of transfer of human medicine must be used. A Norwegian study assessed all 1414 human drug substances marketed in Norway. Of these only 14 were estimated to exceed cut-off values of 100 or 10 µg/kg soil after sludge application. For these substances no PNEC (Predicted No Effect Concentration) values in soil were available. Soil PNEC values for pharmaceuticals were therefore estimated from aquatic PNEC values when available. The estimated soil concentrations of drug substances were low (concentration range 0.01 – 2 mg/kg dry weight (DW)) and well below the estimated PNEC values. The assessment indicated a low increase in human dietary exposure to organic contaminants from sewage treated soil it was opined that this additional exposure constitutes a low risk to the consumers. The Norwegian study also performed an exposure assessment for children to medicine assuming an intake 0.2 g soil per day and compared these intakes with the relevant ADIs or thresholds of toxicological concern (1.5 µg/body weight). It was considered unlikely that consumption of soil mixture added sewage sludge would pose any risk to the children's health. There are only very few attempts to perform consumer risk assessment of the transfer of veterinary medicine to crops, but the risk assessments which have been performed indicate a low risk to the consumer. The concentration of veterinary medicine in the studies, where no assessment has been performed was low, and it was considered unlikely that they would pose a risk to the consumer.

While there is not sufficient data to conclude on consumer risks related to veterinary drugs from animal manure, it is concluded that residues of veterinary and human medicine in sewage sludge are considered of low human health concern.

## II.III Propagation of antibiotic resistance

Antibiotic resistance constitutes a major challenge for public health and the environmental dimensions of antibiotic resistance have lately been widely recognized. Soil bacterial communities even in natural soils are known to harbour an extremely diverse collection of antibiotic resistance genes (ARGs) and other resistance determinants such as mobile genetic elements capable of transferring ARGs from non-pathogenic bacteria to pathogenic bacteria. Hence, agricultural soils constitute a rich source of novel antibiotic resistance mechanisms yet-to-be recruited by pathogenic bacteria. Furthermore, there is now direct evidence that ARG abundance has increased in agricultural soils during the antibiotic era (i.e. since about 1940)

and direct links between bacterial antibiotic resistomes present in agricultural soils and clinical environments have been established.

Animal manure and sewage sludge are thought to comprise major external sources of ARGs in agricultural soils, but no systematic comparative studies of the relative importance of these sources have been carried out. For the purpose of this report we adopted an expert opinion-driven comparative approach in which we aimed to discuss risks posed by sewage sludge deposition to agricultural land by comparing the risks posed by sewage sludge and manure following their amendment to agricultural soils as based on a systematic literature study. Studies exploring these questions in a Danish context are rare. To the best of our knowledge only one Danish study has directly compared the effects of sewage sludge and manure application on antibiotic resistance in agricultural soil. This field study took advantage of the long-term CRUCIAL field trial in Taastrup and used cultivable *Pseudomonas spp* as indicator bacteria. Organic fertilizer amendments corresponding to more than 100 years of application were found to only transiently affect the antibiotic resistance profiles and levels of resistance declined to unfertilized control background levels 9 weeks after application of organic fertilizers. See section 6.1 for further explanation of the field experiment. Other Danish studies have focused on effects of animal manures on antibiotic resistance in agricultural soils. Using a bacterial cultivation based approach, Sengeløv and co-workers were among the first to demonstrate that levels of antibiotic resistance (resistant CFUs relative to total CFUs) increased in farmland soil following manure application, but also that ARG levels quickly decreased to levels similar to unfertilized controls. In a German study Hölzel and co-workers reported a comprehensive comparison of antibiotic resistance levels in three bacterial species (*E. coli, Enterococcus faecalis* and *Enterococcus faecium*) isolated from sewage sludge derived from different sewage treatment plants (n = 111) and liquid pig manures derived from different pig farms (n = 305) in Bavaria. For most tested antibiotics the manure-derived strains displayed a higher frequency of resistance. Multidrug resistance was also most frequent in manure-derived strains. The authors also compared their observed levels of antibiotic resistance to data from the DANMAP survey in Denmark and concluded that sewage sludge antibiotic resistance data were comparable to data from healthy people in Denmark. By contrast, antibiotic resistance levels in German pig manure was higher than corresponding resistance levels in healthy Danish pigs.

The available evidence from the literature indicates that application of sewage sludge does not represent a larger risk than the application of animal manure with regard to dissemination of antibiotic resistance on farmland. Due to the strict requirements in the Danish regulations for land disposal of sewage sludge (Slambekendtgørelsen; Juli 2018) we find it unlikely that application of sewage sludge constitute a significant risk for dissemination of antibiotic resistance to humans, but clearly there is a need for more research to fully justify this conclusion. Immense knowledge gaps on the environmental dimensions of antibiotic resistance

thus precludes a quantitative human health risk assessment due to our limited current understanding of the long-term impacts of manure and sewage sludge fertilization on the underlying ecological and evolutionary processes in soil microbial communities.

# III Risk assessment for the terrestrial environment

## III.I Approach

The application of animal slurry or sewage sludge as fertilizer implies a disposal of a wide range of contaminants to agricultural soils. In the present report, a quantitative assessment of the potential risk for soil-living organisms of four fertilizer scenarios was performed. The scenarios were 1) application of cattle slurry at a rate corresponding to 30 kg P/year, 2) application of pig slurry at a rate corresponding to 37 kg P/year, 3) application of sewage sludge at a rate corresponding to 30 kg P/year, and 4) application of sewage sludge at a rate corresponding to 90 kg P/3 years.

Predicted environmental concentration (PEC) in soil were based on contaminant levels in respective fertilizer matrices reported by the Danish EPA (data on a few compounds, were however of non-Danish origin, due to a lack of inclusion in Danish screenings), and estimated as suggested in the guideline given in the European Chemical Agency's Guidance document on environmental exposure assessment (ECHA, 2016). PEC in soil was calculated after one application and after 10 and 100 years of applications to cover potential accumulation of contaminants following repeated use of the respective fertilizers. Based on the available analyses of Danish slurry and sludge, nineteen metals, 98 organic contaminants, seventeen medical compounds and four estrogens were included in the risk assessment of sludge. In comparison, far less compounds have been (analyzed for and) detected in animal slurry. Five metals, six organic contaminants, seven medical compounds and two estrogens were included in the risk assessment of slurry fertilizers.

A predicted no effect concentration (PNEC) in soil for each included contaminant was adopted from other scientific reports if available, or alternatively calculated from PNEC aquatic or from quantitative structure-activity relationship (QSAR) estimated toxicity endpoint (e.g. EC50).

The final risk of a single contaminant was predicted by a comparison of the estimated predicted environmental concentration with the available predicted no-effect concentration, i.e. PEC/PNEC. However, acknowledging, that one contaminant do not exist in the environment alone, a cumulated risk was calculated for each fertilizer scenario as ∑PEC/PNEC.

### III.II Risk characterizations

#### III.II.I Cumulative risk assessment

The estimations predicted that the cumulated PEC would reach or exceed the cumulated PNEC already after the first application of fertilizer. Application of slurry from cattle and pig resulted in an initial ∑PEC/PNEC of 0.77 and 0.79 whereas application of sewage sludge in the two scenarios resulted in ∑PEC/PNEC of 3.05 and 10.78. Assessing ∑PEC/PNEC after 100 years of repeated application, slurry from cattle and pig resulted in a ∑PEC/PNEC of 2.06 and 8.83 respectively, whereas application of sewage sludge in the two scenarios resulted in ∑PEC/PNEC of 4.89 and 10.78. The difference between ∑PEC/PNEC initially and after 100 years, was markedly larger for slurry fertilizers than for sludge. Slurry fertilizers contain higher concentrations of metal compounds that are not easily removed from the soil, and hence tend to accumulate over time, increasing soil PEC.

To assess the potential long-term exposure to contaminants from slurry or sludge, PEC values in soil six months after application in the 100$^{th}$ year were calculated. After six months the ∑PEC/PNEC of slurry from cattle and pig was 1.42 and 8.23 respectively. Metals accounted for more than 90 % of the summed risk. In respect to sludge the ∑PEC/PNEC after six months were estimated to 2.22 and 3.10 respectively. In these scenarios metals accounted for 72 % and 52 % of the summed risk. Results show that the summed risk of the organic compounds is markedly decreased six months after application.

Generally the ∑PEC/PNEC > 1 is indicating, that there might be a potential risk of adverse effect towards soil-living organisms as a result of application of these fertilizers. The calculated risk refers to the point in time initially after fertilizer application, and hence to the point in time where soil contaminant levels are at their maximum.

#### III.II.II Cattle and pig slurry

With respect to cattle and pig slurry the main contributor of risk are metal compounds, more specifically, zinc and copper. Both metals are used as additives in animal feed and medicines, and in accordance zinc and copper accounted for more than 50 % and 90 % of the summed risk in cattle and pig slurry respectively. The use of pig slurry is estimated to increase natural background concentrations of zinc and copper with approximately 7 and 5 % per year.

From 2022 new regulations will prohibit the use of Zn additives in pig feed, which will reduce the amount of Zn added to agricultural fields, and hence finally reduce the risk of Zn induced toxicity. Similarly Cu will be regulated from 2019 and onwards.

Remaining metals and organic contaminants did not contribute significantly (individual PEC/PNEC < 0.1) to the risk. However, the cumulated risk of organic contaminants (mainly

phenols and PAHs), medical residues (mainly sulfatroxazole) and estrogens reached a ∑PEC/PNEC of 0.94 and 0.85 for cattle and pig slurry respectively, indicating that as a mixture, they may pose a low risk to the soil environment. It should be noted, that slurry from farrowing pigs, however, might result in soil estrogen concentrations above its PNEC.

### III.II.III Sewage sludge

For sludge, the main risk contributors were the organic chemicals. When estimating the ∑PEC/PNEC after 1-10 years of applications, organic chemicals accounted for more 90 % of the summed risk, after 100 years for approximately 70-80 %. The PEC/PNEC was ≥ 0.1 for 12 out of the 98 included organic compounds, these 12 compounds account for 97 % of the calculated risk of organic chemicals. The compounds posing the highest risk in decreasing order are di-n-octylphathalate (DOP) > triclocarban > di(2-ethylhexyl)adipate (DHEA) > nonylphenol-diethoxylate (NP2EO) > tricresylphosphate > triclosan > nonylphenol-monoethoxylate (NP1EO) > LAS > PCA (C14-17) > phenol > PBDE 99. The only single compounds or compound groups with PEC/PNEC > 1 was phthalates (PEC/PNEC = 7.33, with DOP PEC/PNEC = 6.55) and triclocarban (PEC/PNEC = 2.17). Results further indicated that the compounds contributing to the risk are not expected to accumulate in the soil environment.

Phthalates were evaluated to account for 40-50% of the cumulated toxicity of sewage sludge towards soil organisms. Phthalate toxicity in soils is, however, poorly investigated. Previous studies have shown low toxicity of DOP towards soil microorganisms (no significant impact of concentrations up to 500 mg/kg), but soil toxicity information of DOP and DHEA to invertebrates and other soil macro-organisms in soil is lacking from the literature. The PNECs used in the risk characterization were hence estimated from aquatic toxicity and adjusted with an assessment factor of 1000. Phthalates are expected to be fairly rapidly degraded in soils, and based on information of DOP gathered for the present assessment, soil DOP concentrations will reach levels below its PNEC within 27 days of sludge application. Based on the present information on phthalate toxicity towards mainly aquatic organisms, phthalates were evaluated to pose a risk to soil living organisms, especially DOP, in the month immediately after application of sewage sludge. It should, however, be taken into account, that toxicity information is hampered with uncertainties, and hence the present conclusion might be proof of a knowledge gap rather than actual risk.

Triclocarban from sewage sludge was evaluated to contribute with approximately 15 % of the cumulated risk associated with application of sewage sludge. There is to our knowledge no information on actual levels of triclocarban in Danish sewage sludge, and the present PEC is estimated based on concentrations in sludge from an U.S. sewage treatment plant. PNEC was estimated from aquatic toxicity data and corrected with an assessment factor of 1000. Based on the QSAR estimated properties of triclocarban, it is expected that triclocarban is degraded to a concentration below PNEC in the timespan between applications of sludge, even when sludge

is applied yearly. The uncertainties in determination of both PEC and PNEC warrants additional information to improve the risk assessment for triclocarban in Danish soil environments. Based on the present assessment, triclocarban is evaluated to potentially causing harm to soil-living organisms as a result of application to agricultural soils, and hence it is recommended that concentration of triclocarban in Danish sludge should be monitored.

Metal compounds from application of sewage sludge were estimated to reach soil concentrations close to or above the PNEC within the timespan of 100 years (PEC/PNEC after 100 years repeated application of 1.6). Zinc accounted for close to half of the calculated risk of metal compounds. The resulting soil zinc concentrations after 100 years of application were estimated to be approximately ten times lower for sewage sludge than for pig slurry. The natural soil background metal concentration was estimated to increase with less than 1 % per year as a result of application of sewage sludge. The concentration of metal compounds in Danish sewage sludge to be used for soil amendment is closely regulated and monitored. Based on the present results, metals from sewage sludge were evaluated to pose a low risk to the soil environment.

Medical residues and estrogenic compounds were evaluated to have a cumulated risk below 0.5 and hence to pose a low risk to soil-living organisms. The risk evaluation of medical compounds and estrogens in sludge was, however, rendered uncertain due to the lack of knowledge of effects in the soil environment and it was recommended that more studies be performed in order to more accurately characterize the risk of this group of highly bioactive compounds to soil organisms.

## Assessment of the fertilizer potential

Slurry based animal husbandry systems are generally well developed for conserving nutrients for recycling, albeit some losses of gaseous nitrogen are inevitable. By contrast our contemporary sewage treatment systems have not been developed with the aim to recycle, but rather to get rid of unwanted substances in wastewater, in a way that is acceptable in terms of economic and environmental costs. In recent years sewage treatment plants have been developed or retrofitted to specifically retain phosphorus, in order to control feeding of e.g. algal populations in adjacent water bodies. Thus, compared to sewage sludge, the N:P:K ratios of animal slurries will generally come closer to a balanced nutrition of crops, whereas sewage sludge will have far too much P relative to N and K. Thus, only the plants need for phosphorus is fulfilled by the spreading of sewage sludge. Nitrogen in the sewage sludge is found primarily in non-available organic compounds, where less than half are mineralized and can be absorbed by the plants in the first year after the addition. There is therefore a need for supplemental fertilization to meet the needs of mineral N as well as potassium.

## Addressing knowledge gaps

There are a few specific issues that might be relevant to examine, as a consequence of the current assessment.

Ecotoxicological studies involving three or more levels of the soil food web would allow a much greater confidence in determining the effects of di-n-octylphathalate (DOP) and triclocarban, which are responsible for more than half of the predicted toxicity of sewage sludge, but characterized by great uncertainty. A better determination of the ecotoxicological effects could thus allow use of markedly lower assessment factors (e.g. 10 instead of the applied factor of 1000).

These are two examples of ***'known unknowns'*** that we can deal with, and thus increase the confidence in the ecotoxicological risk predictions. However, as stated in the assessment, there is a lack of knowledge of e.g. medicinal residues in animal slurries, and how they impact on soil and human health. Similarly, there may be unknown or uncharacterized compounds in the sewage sludges, and while this assessment has attempted to take cocktail effects into account, it is at best a good approximation of the expected impact on soil organisms that has been given. This introduces the realm of the ***'unknown unknowns'***, which is commonly faced by decision makers when facing complex issues.

We propose that one way of exploring and safeguarding for this, is to take advantage of an existing integrated long-term experiment (CRUCIAL), developed at University of Copenhagen, in which different types of waste and animal fertilizer has been applied in high or even excessive amounts to test if they pose a threat to the ecosystem integrity. A number of studies have emerged from this facility cited in this report, and there are also preliminary studies indicating that i.e. sewage sludge does not impede the health and reproduction of e.g. earthworms and other soil fauna. Indeed the studies indicate that diversity and reproduction rates are high in the sewage sludge treated plots, and so far many other fertility benefits have been recorded from these plots.

To the best of our knowledge this experimental site is unique, and no other place in the world has been developed to this extent, although other relevant points of reference can be found in Europe.

Finally, it needs to be recognized that there is only so much to be done on the basis of scientific studies. An important domain, which is outside the remit of this assessment, is the public acceptability and recognition of the need to recycle resources, that 'in the best of all worlds' would be free of unwanted substances, but in the real world is not.

# IV Conclusions

## IV.I Regarding risks to human health

Based on the review, it is the expert opinion that sewage sludge does not represent a higher risk for propagation and transmission of antibiotic resistance than animal manure. It is not presently possible to quantify the human health risk associated with antibiotic resistance in soil, but we consider it most likely that other transmission pathways (e.g. human-human, animal-to-human or environmental transmission experienced by Danish residents while travelling) may be associated with a higher human health risk.

Among the risk factors discussed, PTEs are the best understood, and Cd and Pb are the most prominent of these in a Danish context, when it comes to direct effects on human health. It would seem highly relevant to further elucidate if the levels of Cd and Pb in crops are indeed on a declining path, due to the much lower combustion related atmospheric emissions over the last decade . Finally, it is considered unlikely that veterinary medicinal residues in pig and cattle slurry are of concern for human health, and it is concluded that veterinary and human medicinal residues in sewage sludge are of low concern.

## IV.II Regarding environmental risks

The presence of compounds in animal slurry and sludge show very little overlap, thus making a direct comparison of the cumulative risk of animal fertilizer and sewage sludge somewhat arbitrary. The lack of overlap is probably due to the differences in origin, but also due to historical differences in the monitoring effort.
The cumulative risk assessment concluded, that there might be a potential risk of repeated use of animal slurry and sewage sludge in all fertilizer scenarios, present in the days initially after application, while pig slurry constitutes a higher chronic risk factor, due to the rather high levels of Cu and Zn.

Based on the low ∑PEC/PNEC it was concluded that organic chemicals, medical residues and estrogens from slurry pose a no or low risk to soil organisms. It should however be noted, that knowledge on organic chemicals in Danish slurry is sparse and hence, though expected to contain less residues from urban uses than sludge, slurry may contain substances not included in the present assessment.

Evaluation of sewage sludge use as fertilizer showed potential toxicity of phthalates and triclocarban. Conclusions are however uncertain due to the lack of both toxicity information, as well as specific Danish measurements of concentrations of some of these compounds. It is hence recommended that these compounds be monitored, at least until further knowledge may discard any uncertainties.

As a final note, it should be mentioned that, as toxicity values of the majority of the organic chemicals towards soil organisms are scarce, values from non-soil organisms or from computational estimations has been used together with large safety-factors (up to a factor 1000). The cumulative risk may therefore be inflated by these uncertainties and the calculations should be verified by experimental data. There are so far no indications from field monitoring studies where contemporary Danish sludge and manure have been used in parallel suggesting adverse effects on the soil biota compared to fields receiving mineral fertilizers.

Overall, it is concluded that sewage sludge from contemporary Danish society does not constitute a higher risk to soil organisms or human health than cattle or pig slurry.

# Table of Contents

# 1 PREFACE

In April 2017, the Organic Business Development Team released a report with 25 recommendations for the Minister of Environment and Food (Det økologiske erhvervsteam 2017). Among these was a recommendation that organic farmers should have opportunities for utilizing nutrients from treated domestic wastewater for nutrient recycling.

A prerequisite for future use of nutrients from treated wastewater is, that quality requirements are met and that application can be explained to (and accepted by) consumers. In partial fulfilment of this, the business team identified a need for a scientific overview of the risks of using nutrients from treated municipal wastewater in relation to other authorized fertilizer sources – e.g. conventional animal manures.

A comparative approach to assess potential risk of using sewage sludge and conventional manures, may usefully inform future decision makers in the regulation of organic farming systems.

Dependent on the result of the scientific investigation, the Organic Business Development Team foresaw that Denmark could chose to work to expand Annex 1 of the EU Ecology Regulation, to allow the organic farmers to use nutrients from municipal wastewater or other acceptable derived sludge products. Mobilization of support for this should be done by the Ministry of Environment and Food in collaboration with the Organic Farming Industry.

Thus, based on available literature, this report aims at creating an overview of the content of following compounds in pig and cattle slurry as well as sewage sludge:

1. The fertilizer potential of the resources
2. Heavy metals
2. Persistent organic pollutants
3. Residues from veterinary and human medicines
4. Female estrogens
5. Antibiotic resistance genes

Based on these, the report will make a qualitative assessment of risks for humans and for the surrounding soil environment after the application of pig and cattle slurry as well as sewage sludge to agricultural soil.

# 2 INTRODUCTION

## 2.1 Background

Phosphorus (P) is an essential plant nutrient and it is common practice to apply P-rich fertilizers to agricultural soils in order to maximize yields. P is however a nutrient with finite resource regarded as a 'high supply risk" substance by the European Commission and was in 2014 added to the list of 20 critical raw materials essential for production in Europe (European Commission 2014), and hence efficient utilizations of organic P sources are pivotal for preserving P as a recyclable nutrient in agricultural systems. In line with this, the European Commission recommends that sewage sludge, which is especially rich in P, should be recycled to farmland to the extent it does not pose a risk to the environment and health. Also in Danish policy it is favoured to use sewage sludge as fertilizer. The national resources strategy 'Danmark uden affald' on the use of waste for agricultural purposes in Denmark states that as much waste as possible must be recycled. At the time of this writing around 75% of the Danish sludge is recycled to agriculture, according to the Danish Business Association for Biomass Recycling (Justesen and Nielsen 2014).

Our contemporary society depends on a large range of organic chemicals. Of the 50 million chemicals entered in the Chemical Abstracts Registry, approximately 143,000 chemicals are registered at the European Chemicals Agency for industrial use. Chemicals that are commonly used may be counted in 10s of thousands. Some of these will ultimately enter our wastewater treatment plants, and, depending on the intrinsic properties of the substances and the technical specification of the wastewater treatment plants, these may end up in sewage sludge.

To ensure the quality of sludge to be used as fertilizer, samples are analysed for the presence of a range of listed substances, summarized in Table 2.1.

In contrast to the rather strict regulations and control for sewage sludge quality, the application of manure is regulated through the national maximum P and N application rates. In the Danish regulation these are set to 170 kg N/ha and 30-43 kg P/ha respectively, the latter depending on the type of manure used (Miljø- og Fødevareministeriet 2017b).

In practice the produced amount of animal slurry is much higher than the amount of sludge. This in part explains the differences in quality analyses performed. The smaller amount of sludge, allows for a strict control and for disposal when quality do not meet the set criteria, which is not possible with the large amounts of animal slurry.

**Table** 2.1 Quality criteria for Danish sewage sludge to be applied as fertilizer to agricultural soils. Levels are cut-of values. For selected metals, the cut-of values can be assesses either per kg dm or per kg total P, for the remaining only per mg/kg dm. Levels must be below either of the given values (modified from Miljø- og Fødevareministeriet 2017a).

| **Metals** | **mg/kg dm** | **mg/kg total P** |
|---|---|---|
| Cadmium (Cd) | 0.8 | 100 |
| Mercury (Hg) | 0.8 | 200 |
| Lead (Pb) | 120 | 10000 |
| Nickel (Ni) | 30 | 2500 |
| Chromium (Cr) | 100 | – |
| Zinc (Zn) | 4000 | – |
| Copper (Cu) | 1000 | - |
| **Organic compounds** | | |
| LAS | 1300 | - |
| PAH (11) | 3 | - |
| NPE | 10 | - |
| DEHP | 50 | - |

dm:dry matter; LAS:linear alkylbenzenesulfonates; PAH: polyaromatic hydrocarbons,; NPE: nonylphenol + nonylphenol ethoxylates; DEHP: di(2-ethylhexyl)phthalate.

Farmyard manure is not likely to contain such a wide range of organic compounds as sewage sludge (NOVANA, 2015). Instead the main concern is the content of heavy metals, originating from feed additives, and residues of veterinary medicines. Additionally, hormones excreted by livestock have caught the attention of human and environmental risk assessors (Kjær et al., 2007). Finally multi-resistant pathogens in animal slurry has gained increasing interest and concern, as is discussed in detail in the section on assessment of risks to human health.

In Denmark, as in the EU, the use of sewage sludge is not permitted in organic farming, while the use of manure from conventional farms is allowed (European Union, 2008). The aim of the present report is to perform a comparative assessment of the risks associated with use of sewage sludge and conventional slurry from cattle and pigs as fertilizer sources in agriculture. Focus is partly on the environmental effects, more accurately on soil-living organisms and partly on the potential risks to humans. The latter however, is only assessed qualitatively. The risk to the soil environment is performed quantitatively and follows the principles of cumulative risk assessments (Hardy et al. 2018).

## 2.2 Essential differences between sewage sludge and animal slurries

Some essential differences between sewage sludge and animal slurries may be illustrated by considering the nitrogen flow from food or feed intake – to the return to land (Figure 2.1).

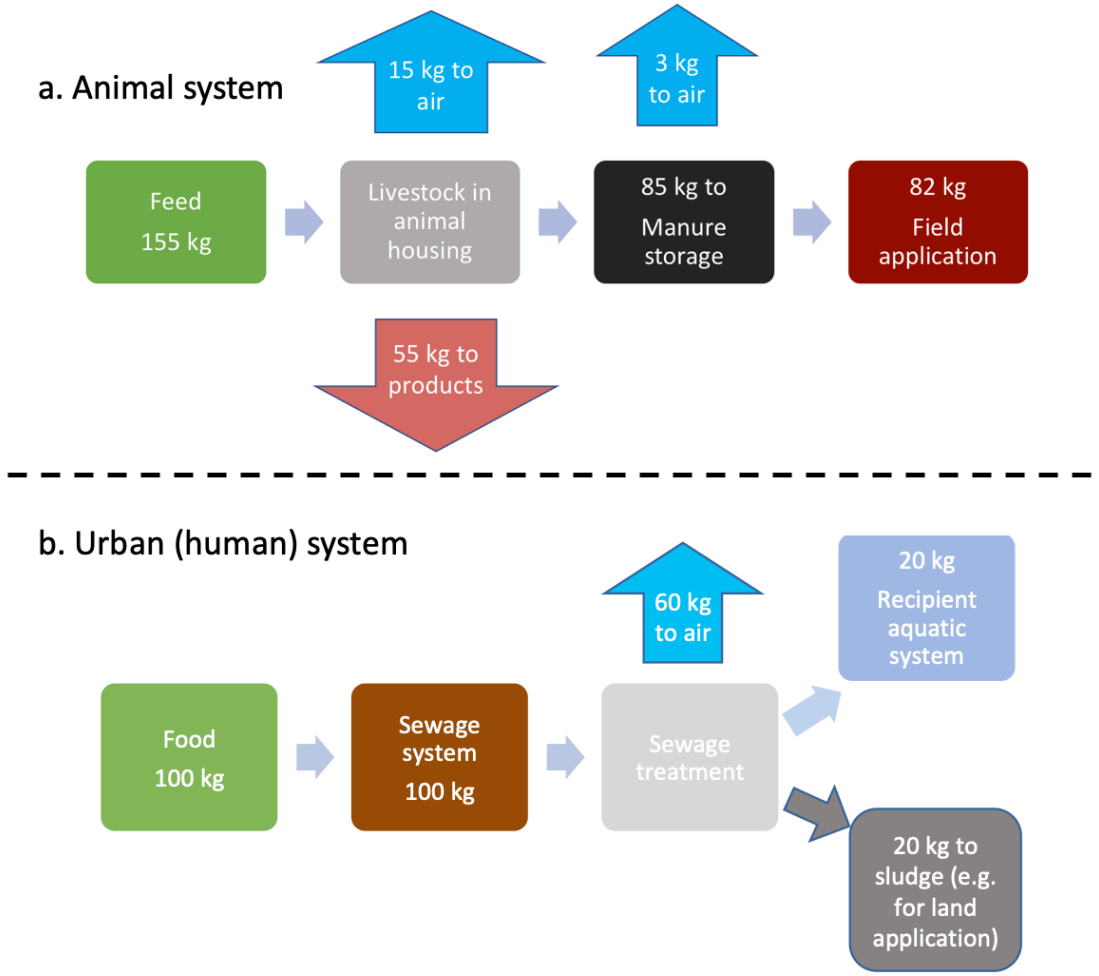


**Figure** 2.1. Principal flow of nitrogen (N) in the human and animal systems, assuming that there is no net uptake of nitrogen in the human system. Adapted from Magid et al, 2006 and Jarvis et al, 2011.

It is fair to state that our contemporary sewage treatment systems were not developed with the aim to recycle, but rather to get rid of unwanted substances in wastewater, in a way that is acceptable in terms of economic and environmental costs. This is reflected in the substantial atmospheric emission of mainly $N_2$ (non-reactive nitrogen) occurring through denitrification, but also the loss of N and other soluble components (nutrients as well as other chemicals) to the downstream recipient aquatic environment. An exception is phosphorus (P), where in recent years a targeted retention in the sludge has been applied, in order to control the emission of P to surface waters. This retention is brought about either through biological removal, a combination of biological and chemical removal (using iron or aluminium based flocculants, or a purely chemical retention approach. By contrast, animal production systems have been developed that conserve much more nitrogen as well as principally all soluble and less soluble

components (nutrients and other chemicals), and efforts are made to devise systems that further minimize atmospheric emissions from housing and storage systems.

The substances retained in the sewage sludge are thus generally less water soluble. From prior literature reviews and risk assessments (see below) it may be inferred that the most toxic substances (e.g. tetrachlorinated-p-dioxin) cannot be detected in the sludge. Undesirable substances in sludge are affected by a number of mechanisms that inhibits or prevents transfer to crops and the food chain in general, including: (i) rapid evaporation and loss to the atmosphere for some substances, (ii) the rapid biodegradation and minimal or no persistence for others, (iii) strong adsorption of persistent connections to the earth, and (iv) minimal or no uptake by plants and grazing animals.

## 2.3 Some recent reviews and risk assessments concerning sewage sludge

### 2.3.1 Risk assessment of contaminants in sewage sludge applied on Norwegian soils

A comprehensive Norwegian study assessed the risk of using sewage sludge as fertilizer and soil conditioner in agriculture and using derived products in public parks and private gardens (Eriksen et al. (2009). An assessment was made of the potential risk of dispersal of sewage sludge for soil living organisms, the aquatic environment, grazing animals, animals eating feed based on plants from sludge-treated soil, children eating soil, and humans consuming drinking water, crop plants and/or meat affected by the use of sludge as soil conditioner.

A risk assessment of all these exposure routes was made for the following contaminants:

Heavy metals: cadmium (Cd), phthalates (diethylhexyl phthalate DEHP, Dibutyl phthalate DBP), lead (Pb), mercury (Hg), nickel (Ni), zink (Zn), copper (Cu) and chromium (Cr)

Xenobiotic Organic Contaminants: octylphenols and octylphenol ethoxylates, nonylphenols and nonylphenol ethoxylates, linear alkylbenzenesulfonates (LAS), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs).

Furthermore, the study evaluated the risk associated with a range of pharmaceuticals. The predicted environmental concentrations (PECs) in soil, as well as human and animal exposure to the contaminants following the use of sewage sludge as soil conditioner were estimated by use of mathematical modelling based on the guidelines given in the European Chemical Agency's Guidance document on environmental exposure assessment (ECHA, 2016). The risk assessment covered evaluation after one application and the potential accumulation of contaminants following repeated use of sewage sludge in a 100-year perspective.

The estimated predicted environmental concentration for each contaminant was compared with the available predicted no-effect concentration (PNEC) for soil. For heavy metals the model showed that no metal would reach the PNEC values within the timeframe of 100 years. Consequently, it was considered that metals in sludge constituted a low risk to soil living organisms. However, the model estimates indicated that the soil concentrations of Cd, Hg, Cu and Zn, and partly also Pb would increase following repeated use of sewage sludge. Cadmium and Hg, as well as Pb are of particular concern due to their inherent toxic properties and an increase is undesirable even if the soil concentration remains below the PNEC values.

Octylphenols, nonylphenols and LAS were the only contaminants where the PEC exceeded the PNEC. However, these are rapidly degradable substances (t1/2 in soil = 8-10 days) where the highest concentrations were found immediately after application of sewage sludge followed by a rapid decrease. Taking into account the uncertainties related to the occurrence levels, and the rapid degradation in soil, it was considered that octylphenols, nonylphenols and LAS are of low concern. Only a few PAHs and PCBs were expected to accumulate with repeated use of sewage sludge over a 100-year period and the model indicated that the concentrations of these substances would be well below the PNEC value even at the end of the 100-year period.

All the assessed organic contaminants were found to constitute a low risk to the soil environment.

Of the more than 1400 drug substances sold in Norway, only 14 were estimated to exceed cut-off values of 100 or 10 µg/kg soil after sludge application. For these substances no PNEC values in soil was available. Soil PNEC values for pharmaceuticals were therefore estimated from aquatic PNEC values when available. The estimated soil concentrations of drug substances were low (concentration range 0.01 – 2 mg/kg dry weight (dw)) and well below the estimated PNEC values. Thus drug substances in sewage sludge were assessed to constitute a low risk for soil-living organisms.

The potential transfer to the aquatic environment of metals, organic contaminants and drug substances from sludge applied within the boundaries set by regulatory statutes was assessed to be of no significance.

The risk of adverse effects in farm animals grazing on or receiving feed from sewage sludge treated areas seems to be negligible for a range of contaminants. However, considering use of sewage sludge directly on grazing areas without ploughing lead might be an exception and may constitute a risk in young animals.

The human dietary intakes via the different exposure routes assessed were combined – i.e. drinking water, plant and animal derived food products. The estimated concentrations of

contaminants in soil indicate that repeated application of sewage sludge on a field during a 100 year time period will lead to an increase in soil concentrations of certain heavy metals such as Cd and Hg. A consequence of this accumulation in soil may result in an undesirable increase in human dietary intake of particularly Cd, but also Hg. However, Eriksen et al. (2009) estimated that the increase in intake of metals from animal-derived food products or drinking water as a consequence of use of sewage sludge as fertilizer to be very low (<5 % of estimated current total intake) and thus of low concern. The organic contaminants addressed in the Norwegian risk assessment are either degraded in the soil or poorly absorbed in plants. The assessment therefore indicated a low increase in human dietary exposure to organic contaminants from sewage treated soil and it was opined that this additional exposure constitutes a low risk to the consumers.

It was deemed unlikely that antibacterial resistance may be promoted in the sewage treatment plant (STP) water, in the sludge or in the soil following application of sewage sludge as fertilizer. An exception might be a potential development of resistance to the fluoroquinolone ciprofloxacin in soil due to its persistence.

The risks were assessed chemical by chemical, since no methodology for the risk assessment of the mixture occurring in sewage sludge was available. Most of the estimated exposures were well below any predicted effect concentration, making any interaction less likely, unless the contaminants have the same mode of action.

### 2.3.2 A review of organic contaminants in sewage sludge (biosolids) and their significance for agricultural recycling

Smith (2009) reviewed the concentration data for organic contaminants (OCs) in sewage sludge and assessed the consequences and significance of OCs for the environment, human health and the food chain when sewage sludge is recycled to farmland as a fertilizer. He notes that according to the European Commission there are no recorded cases of human, animal or crop contamination due to the use of sludge on agricultural soils following the provisions of Directive 86/278/EEC. Despite the international support for recycling sludge to land, the acceptance of this practice among different European countries varies considerably and has declined markedly in some cases. For example, concerns about the potential consequences for human health and the environment of potentially toxic substances and harmful micro-organisms have led to the banning of the use of sludge in agriculture in Switzerland, despite official recognition that there is no conclusive scientific evidence that the practice is harmful in any way. Smith (2009) analyses available data on bulk-volume and industrial compounds, as well as endocrine disrupters, pharmaceuticals, antibiotics and personal-care products.

#### *2.3.2.1 Human health risks*

It is found that OCs present minimal risk to the human food chain from land application of sewage sludge. Based on the analytical evidence the most toxic compounds (e.g. Tetrachlorodibenzodioxin) cannot be detected in sludge. These compounds are also influenced by a variety of mechanisms that prevent transfer to crop tissues and the human food chain, including: (i) rapid volatilization and loss to the atmosphere; (ii) rapid biodegradation and minimal or no persistence, or (iii) strong adsorption of persistent compounds.

The risk to human health via dietary intake of OCs from crops grown on sludge treated soils is minimal owing to the absence of crop uptake. In recent years the potential impacts on the food chain of persistent OCs in sludge, including PAHs, PCDD/Fs or PCBs, have been a key concern for agricultural utilization. However, international emission controls on the main point sources of these priority-persistent compounds have significantly reduced their entry into the environment and consequently also into the urban waste water collection system. Thus, atmospheric deposition and environmental cycling are the main sources of PCBs in sludge, and consequently the concentrations of this historically used chemical in sludge generally represent background environmental levels.

#### *2.3.2.2 Crop yields and soil fertility*

Smith (2009) found no evidence that the vast majority of sludge-borne OCs have a detrimental impact on crop yield or soil microbial processes. Earlier concerns about the potential impact of LAS, a detergent surfactant present in large concentrations in sludge, on soil ecological processes have been further elucidated and shown to be unfounded. While the presence of large concentrations of certain high-volume bulk chemicals, such as LAS, warrants careful investigation and assessment of the risks to the environment when sludge is used as an agricultural soil amendment, this does not necessarily represent a risk to the soil ecological environment.

Phthalates were not found to cause any significant adverse effects on soil microbial processes or on soil fertility. In general, high-volume usage compounds have very low toxicity and degrade rapidly in soil. A number of emerging compounds were identified in this review as having a potential impact on soil microbes and these belong to the group of chemicals described as body-care products, e.g. triclosan, and the significance of these warrants further investigation.

Despite the extensive range of organic chemicals that can be present in sewage sludge, the expanding experimental evidence base (147 papers in this review) indicates that these are not a significant limitation to the agricultural use of sewage sludge. This view is based on a technical evaluation of the situation, which acknowledges that the presence of effective source control measures and small concentrations of persistent contaminants in sludge, biodegradation and behaviour in soil, absence of crop uptake and sludge application practices minimize the potential impacts of OCs in sludge on soil quality, human health and the environment.

According to Smith (2009) the consensus view therefore is that there appears to be no scientific rationale for including numerical limits on OCs in quality assurance systems for the agricultural use of sewage sludge. Furthermore, the chemical quality of sludge is continually improving and concentrations of potentially harmful and persistent organic compounds have declined to background values. Thus, recycling sewage sludge on farmland is not constrained by concentrations of OCs found in contemporary sewage sludge. A number of issues, while unlikely to be significant for agricultural utilization, require further investigation and include: (i) the impacts of chlorinated paraffins on the food chain and human health, (ii) the risk assessment of the plasticizer di(2-ethylhexyl)phthalate, a bulk chemical present in large amounts in sludge, (iii) the microbiological risk assessment of antibiotic resistant micro-organisms in sewage sludge and sludge-amended agricultural soil, and (iv) the potential significance of personal-care products (e.g. triclosan), pharmaceuticals and endocrine-disrupting compounds in sludge on soil quality and human health.

### 2.3.3 Review of 'emerging' organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids

The author of the aforementioned paper, later on published a review specifically on 'emerging' organic contaminants (Clarke and Smith, 2011). Of the 50 million chemicals entered in the Chemical Abstracts Registry approximately 143,000 chemicals are registered with the European Chemicals Agency for industrial use. Clarke and Smith (2011) identified research and monitoring priorities based on the following 5 criteria: **1.)** environmental persistence in soil environment (>6 months); **2.)** potential for human health impacts resulting from the land application of biosolids; **3.)** evidence or likelihood of bioaccumulation in humans or the environment; **4.)** evidence of ecotoxicity, and **5.)** the quality of empirical data and trends on the contaminant in biosolids internationally.

They found that two chemical classes warrant particular note. These are the perflourinated chemicals (PFCs) and polychlorinated alkanes (PCAs). PFCs are an emerging environmental concern as they have been detected in human blood and environmental samples throughout the world. They have a unique chemistry for a chemical defined as a persistent organic pollutant (POP) that facilitates a degree of water solubility, and therefore, there is an increased likelihood of exposure through all pathways (water contamination, plant accumulation and grazing animal accumulation) compared to other POPs. PCAs were found at relatively high concentrations in sludge (mean concentration 1800 mg $kg^{-1}$ dw). Comparison of the concentrations of these compounds to PCBs and PCDD/Fs shows that the PCA content in sludge is three orders of magnitude higher than PCB values for instance, and signals the importance of further investigations into the significance of PCAs in biosolids for land application. While recycling biosolids on land is recognised internationally as the most sustainable option for managing the residual sludge from urban wastewater treatment, continued vigilance in assessing the

significance and implications of ‘emerging’ OCs in sludge was deemed necessary to support and ensure the long-term sustainability of this management option.

# PART I

# 3 FERTILIZER POTENTIALS

## 3.1 Nutrient utilization and soil fertility building value

In order to understand the fertilization value of sewage sludge, relative to cattle or pig manure, it is important to recognize the differences in recycling efficiency, that was touched upon in section 2.2, and is illustrated in Figure 3.1 below. The recycling efficiency is defined here as the amount (%) of nutrient recycled, compared to that excreted from animals or humans.

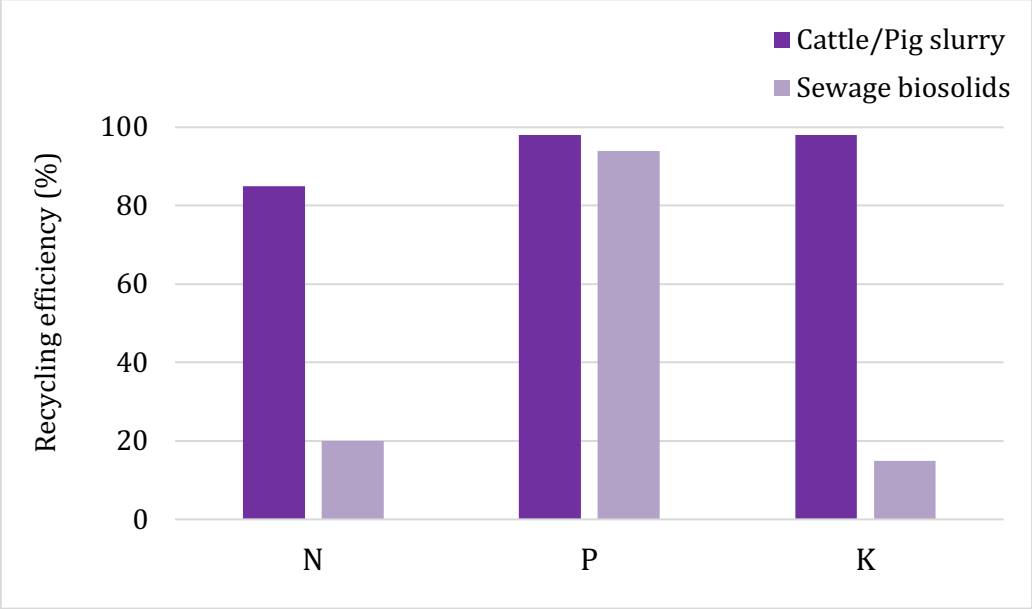


**Figure 3.1**. Recovery of selected macronutrients in the biosolids/sludge from sewage treatment systems (based on Magid et al., 2006) compared to the recovery of macronutrients from animal slurry from husbandry systems (based on Jarvis et al., 2011).

As discussed previously, slurry based animal husbandry systems are generally well developed for conserving nutrients for recycling, albeit some losses of gaseous nitrogen are inevitable. By contrast our contemporary sewage treatment systems were not developed with the aim to recycle, but rather to get rid of unwanted substances in wastewater, in a way that is acceptable in terms of economic and environmental costs. Thus, compared to sewage sludge, the N:P:K ratios of animal slurries will generally come closer to a balanced nutrition of crops, whereas sewage sludge will have far too much P relative to N and K.

## 3.2 Crop utilization of nutrients from waste materials

Utilization of nutrients in waste products supplied to crops is important both in order to minimize losses to the environment and to give the farmer a financial benefit from spreading these to the soil. Generally, sewage sludges and other waste products introduced within the

framework of the waste directive cannot fully cover the needs of crops, due to the unbalanced contents of macronutrients, relative to crop demand, that arises due to losses of N and other nutrients relative to P. Thus, there is a need for supplemental fertilization. Therefore, the crop demand and utilization of specific nutrients is also important in order to determine the amount of additional nutrients to be supplied to the soil in form of mineral fertilizer. If more nutrients are added than the plants can utilize, the nutrients can be transported to the aquatic environment where they may pose an environmental risk.

### 3.2.1 Nitrogen

Sewage sludge contains relatively large amounts of nitrogen (N), but the majority is bound in organic compounds. Therefore, the conversion / mineralization of the organic part of the sludge is an important factor in releasing plant-accessible nitrogen in the form of ammonium or nitrate.

According to Epstein (2003) anaerobically treated sewage sludge can contain between 5 and 176 kg total N / ton of dry matter. In 2002, Danish wastewater sludge comprised between 30 and 60, an average of 44.4 kg total N / ton of dry matter (Miljøstyrelsen, 2017a). The proportion of this inorganic form ($NH_4^+$ and $NO_3$) varies, but will typically only be between 10-20% of the total nitrogen content for anaerobic treatment (Epstein 2003; Petersen 2003; Petersen et al. 2003). Counting sewage sludge and manure in total, agricultural land can only be supplied with 170 kg of total N per ha per year.

The major difference between nitrogen forms in slurries and sewage sludge is the much higher proportion of available N ($NH_4$-N) in the animal products, but also higher potential loss upon application. This has been extensively reviewed in the literature, e.g. Jarvis et al. (2011)

### 3.2.2 Phosphorus

Phosphorus (P) is found in sludge primarily as inorganic phosphate ($PO_4^{3-}$). In the treatment processes of sewage, iron or aluminium salts are often added, whereby phosphate precipitates into complex more slowly soluble components in the sewage sludge.

Phosphate associates strongly to the soil matrix, either with calcium or aluminium and iron oxides, and as a result, P, which is soluble immediately upon addition to soil, will become increasingly less soluble, until with time an equilibrium solubility level will be found. Conversely, when soil solution is depleted by plant roots, the soil will be able to replenish the soil solution, until the soil is gradually depleted for its P resource.

Recently, it has been shown that solubility of triple superphosphate added to soil rapidly decreased (over a few weeks), the phosphate from sludge provided by the Avedøre wastewater treatment plant (biological removal in combination with Fe-flocculation) became increasingly

soluble over time (Lemming et al., 2017). This was also the case for ashes derived from incineration of the this source, albeit at a lower level of solubility. Thus, it can be argued that sewage sludge may function as a slow release fertilizer, and perhaps with similar or even higher long-term efficiency than the standard soluble commercial P fertilizers.

Moeller et al. (2018) reviewed reports of a range of alternative P fertilizers, and reported the following P average efficiencies compared to water soluble P fertilizer for the first growing season:

1) sewage sludge - biological P removal, 90% (54 data points), 2) chemical P removal, 60% (126 data points), 3) untreated ashes from sewage sludge, 30% (31 data points), 4) ASH-DEC®$^{\delta}$ Mg-treated ashes 45% (52 data points), 5) Animal manures (broadly) 105% (110 data points).

It should be noted that alternative fertilizers in many experiments have been shown to give rise to more P uptake than the standard reference soluble P fertilizer, albeit the average values per category are usually below 100%. This is presumably due to the fact that more slowly available fertilizers may work better than the highly soluble form, since the phosphorus is rapidly absorbed to soil particles, becoming less available within a rather short timespan. Thus even first year effects measurements may show a beneficial effect of slow release fertilizer.

According to the Danish regulation on waste (Miljø- og Fødevareministeriet, 2017a), 30 kg P / ha / year may be applied as an average per year waste water sludge and e.g. composted waste, comprising up to 90 kg P in a onetime delivery that would cover 3 years of P fertilization.

As of January 2017 new rules for Danish agriculture has led to limitations in the amounts of P that may be applied to land annually, ranging from 30-43 kg P $ha^{-1}$, dependent on the type of manure or compound fertilizer used.

In the present assessment we estimate the ecotoxicological risks based on the assumption that 30 kg P $ha^{-1}$ annually is added with either sewage sludge or cattle slurry or 37 kg with pig slurry, or as an alternative 90 kg P $ha^{-1}$ with sewage sludge every third year. These numbers correspond to the maximum limits set by current Danish legal standards (Miljø – og Fødevareministeriet 2017 a and b).

### 3.2.3 Potassium

Potassium (K) is absorbed by the plants as $K^+$. There is generally a low potassium content in sewage sludge and in 2002 the average sludge content was 2.1 kg K / ton of dry matter (Miljøstyrelsen 2004b). The reason for the low content is that the potassium compounds in the wastewater are mainly present in the soluble form and thus not recovered in the treatment

process. Therefore only a small amount will be found in the sludge after the separation processes. Potassium is present in much more adequate amounts in animal manures. To the best of our knowledge there is equal plant availability of potassium present in sewage sludge and animal manures.

### 3.2.4 Calcium

Calcium (Ca) is also an essential macronutrient for plants, but is usually found in sufficient quantities in Danish arable soils that are regularly applied with calcite or contain naturally occurring lime. Wastewater sludge contains only significant amounts of calcium if the sewage sludge is stabilized in post-treatment by adding quicklime for stabilisation. This practice is not so common in Denmark, only 4% of all sludge is treated with lime (Miljøstyrelsen 2004b), but it is more prevalent in other countries, for example in Norway.

### 3.2.5 Micronutrients

In addition to the three above-mentioned macronutrients (N, P and K), the plants also need a number of micronutrients. In respect to sewage sludge there are usually seven micronutrients mentioned. These are boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), Nickel (Ni) and Zinc (Epstein 2003). These nutrients are found in very small amounts in the soil (trace elements). The plants therefore also only need these substances in small quantities, and for most of the substances, increased concentrations in the soil are toxic to the plants. Some of the substances are even subject to statutory regulation such as heavy metals (Cu, Ni, Cr and Zn).

### 3.2.6 Other factors

The supply of wastewater sludge not only affects the soil via increased nutrient supply, but also leaves clear traces on the physical properties of the soil. With increased amount of sewage sludge added, soil density decreases while porosity and typically also the content of organic carbon increases. In addition, the soil exhibits increased water retention capacity (Samaras et al., 2008).

With an average content in Danish wastewater sludge of approx. 40 kg P / ton of dry matter, the maximum amount will be 0.75 tonnes of dry matter/ha/yr. As a result of both practical and cost-related reasons, one would usually choose every 3 years to add the triple dose, i.e. 2.25 tons of dry matter/ha.

As follows from the discussion above, only the plants need for phosphorus is fulfilled by the spreading of sewage sludge. Nitrogen in the waste water sludge is found primarily in non-available organic compounds, where less than half are mineralized and can be absorbed by the

plants in the first year after the addition (Epstein 2003). In most cases, there is therefore a need for supplemental fertilization to meet the needs of mineral N as well as potassium.

# PART II

# 4 HUMAN RISK ASSESSMENT

Based on our current understanding of the major concerns to human health, we have decided to focus on issues related to potentially toxic elements, residues of veterinary and human medicine, and finally propagation of antibiotic resistance. This is a qualitative approach, based on the state of the art in the literature.

## 4.1 Potentially toxic elements (heavy metals)

Potentially toxic elements (PTEs) are typically identified as zinc (Zn), copper (Cu), nickel (Ni), cadmium (Cd), lead (Pb), mercury (Hg), chromium (Cr), molybdenum (Mo), selenium (Se), and arsenic (A . These elements occur naturally in many soils, generally in low non-toxic concentrations, however, accumulation of these elements in soils as a result of application of manures, slurries and waste products are of major concern. We consider the potential for plant uptake, and thus the ensuing transmission to humans vie food in the following. Antoniadis et al. (2017) assessed the soil-to-plant availability index (transfer coefficient, TC), because it encompasses all soil and plant factors related to PTE phytoavailability. While all these elements may show a bioconcentration factor >>1, it is of critical importance to recognize that a major barrier to their entry into plants is the soil condition, and crucially the management decisions that are made during agricultural production.

Soil pH is the single most important factor affecting PTE phytoavailability. For cationic species, lower pH values result in higher mobility and thus availability (Lee et al., 2009; Brokbartold et al., 2012), while the opposite is true for anionic species (Kader et al., 2016). Furthermore, when PTEs are introduced to soils, they undergo transformations associated with (and dependent upon) soil colloids. Thus, PTEs over time may be retained in a less-reversible manner onto interlayer clay sites, especially when blocked by lattice-fixed cations such as $K^+$ or occluded by evolved Fe or Al polymers. Over time, PTEs may even be engrafted during clay crystallization procedures with isomorphic substitution into mineral structures.

For Danish conditions this implies that a control of soil pH to near neutral will effectively reduce the plant uptake of Cd, which is the main element of concern in our context. This may be illustrated with results from the long-term agronomic experimental site on waste recycling established by Copenhagen University (CRUCIAL). López-Rayo et al. (2016) found that long-term amendment of urban and animal wastes equivalent to more than 100 years of legal application had minimal effect on plant uptake of potentially toxic elements, which is in agreement with the Norwegian risk assessment by Eriksen et al. (2009). See section 6.1 for further explanation of the field experiment. Of the elements studied, only Zn and Cu were significantly elevated in soils receiving urban waste treatments. In oat grain Cd was significantly elevated in a sewage sludge treatment corresponding to more than 200 years legal application, but even in this extreme case the concentration of Cd in grain did come close to

the relevant EU limit for Cd content. In pea plants the concentrations of Zn and Mo were significantly higher in plants grown in soil that had received large amounts of urban wastes compared to an unfertilized control. The build-up of Zn could be regarded as a beneficial side effect of using sewage sludge as a fertilizer due to the modest increase in the Zn concentrations and the status of Zn as an essential element. In several cases the effect of adding urban and animal wastes resulted in a decrease of PTE concentrations in plants relative to a control soil that was unfertilized since 2003 in the CRUCIAL experiment. Thus, both Cd and Ni concentrations in oat grains were reduced by amendments with household waste compost, while Ni concentrations also decreased upon fertilization with NPK, Deep Litter, Cattle slurry and sewage biosolids amended in an amount equivalent to about 100 years of legal application. Organic matter added to soil may decrease PTE bio-availability by affecting their solubility in soil. This has been shown repeatedly in long-term field studies where organic residues have been applied, not only due to the retention capacity of organic matter for PTEs (Cambier et al., 2014), but also due to the fact that organic matter application increases plant biomass production and thus gives rise to a 'dilution effect' (López-Rayo et al., 2016). It should be noted that the adsorption of Cd and other cationic PTE's is reversible and when pH changes over time the binding will change. If agricultural soils are not limed, most likely pH will decrease over time and e.g. Cd availability increase over time

### 4.1.1 Comparative aspects and recycling dilemmas

Undoubtedly PTEs pose risks that cannot be ignored in that they are absolutely non-degradable, and therefore potentially available once applied, should soil conditions develop in an undesirable way (e.g. pH lowering due to acidification). In the context of the current writing the main interest is the comparison between sewage sludge and cattle and pig slurry. The knowledge base on PTEs in contemporary Danish sewage sludge needs updating. As far as we know, values that are measured to meet quality control standards are reported regularly to the Ministry of Environment and Food, but this information has not been made available.

Cd has long been recognized as a major health threat to humans, as it represents one of the most toxic substances released into the environment (Clemens & Ma, 2016), and thus in principle any increase of Cd intake should be avoided. However, as seen from the discussion above, fertilization with organic wastes may give rise to decreases in Cd concentrations of plant materials due to various mechanisms. In addition, there is a need to recycle waste materials, and especially to conserve phosphorus, which is a critical and finite resource in the emerging bio-economy (Staffas et al., 2013). Furthermore, the uptake of Cd and other cationic metals can be much reduced by liming, ensuring that soil pH is close to neutral (but see discussion above). In the Norwegian risk assessment on sewage sludge (Eriksen et al., 2009) it is considered that food produced from soil amended with sludge for 100 years would increase Cd intake per capita with less than 5% relative to the baseline, and this is deemed acceptable. However, more

recently a discussion has started to play out, based on the assertion that Cd concentrations in food produced in Europe may be generally declining, but see below. If this is the case – it becomes pertinent to ask – how quickly such concentrations should decline, considering the need to recycle finite resources?

### 4.1.2 Are European-wide soil and food concentrations of Cd declining?

Pacyna et al. (2009) published a study of changes of emissions and atmospheric deposition of mercury, lead, and cadmium, in which they assessed the effects of the implementation of various strategies of emission controls in Europe. They found that substantial declines of emissions of mercury, lead, and cadmium were related to the reduction trends of air concentrations of these metals during the last 2 decades. Their assessment indicates that cadmium and mercury emissions have been reduced by a factor 5, while lead emissions is reduced by a factor 15. This is of substantial interest, since the current regulation was founded in a historical period where atmospheric deposition to land was much higher than the deposition today.

Based on this, Smolders (2013) revisited and updated the effect of P fertilizers on cadmium accumulation in European agricultural soils. Future long-term changes (100 years) in soil Cd concentrations were calculated for four fertilizer Cd concentrations (20, 40, 60 and 80 mg Cd per kg $P_2O_5$) and for 2160 different scenarios covering the range of conditions encountered in the EU, i.e. P fertilizer use, soil properties (pH, organic carbon content), climatic conditions affecting leaching, type of crop and atmospheric deposition (zero to worst case); and for the European average values. For the sake of comparability with Danish fertilizer regulation where 100 mg Cd per kg P is the upper regulatory limit for P-fertilizer, we recalculate the above Cd concentrations (20, 40, 60 and 80 mg Cd per kg $P_2O_5$) to being equivalent of 46, 92, 138 and 183 mg Cd per kg P.

The following conclusions could be drawn:

- At the highest Cd concentration studied, 183 mg Cd per kg P, soil Cd is predicted to remain rather constant.
- At 138 mg Cd per kg P, soil Cd is predicted to change by -7% in 100 years, i.e. a net decrease.
- At 92 mg Cd per kg P, these values are -14% and at 46 mg Cd per kg P they are -20%.
- Thus the current average EU Cd mass balance is negative compared to positive balances estimated in 2002.

Thus the dilemma between recycling of a possibly slightly Cd tainted urban waste resource and of ensuring the least possible negative impact on human health may be stated in the following terms:

There is a lack of accessible information on the Cd content of contemporary Danish sewage sludge, which should be remedied by ensuring access information routinely reported to the ministry of environment and agriculture. Most likely the sewage sludge will have higher content than animal slurries, and thus may potentially contribute to a relative increase in human dietary Cd intake. This may be controlled, however, by ensuring that the soils receiving these wastes have a pH close to neutral. Furthermore, it seems likely that concentrations of Cd in agricultural soils are declining, even if P fertilisers with up to 100 mg per kg P are being used. This should be further examined and verified. If this is indeed the case – it becomes pertinent to ask – how quickly such concentrations should decline, considering the need to recycle finite resources of phosphorus, such as sewage sludge?

Overall, we conclude that there is a low risk connected to PTE's in connection with human intake of crops fertilized with Danish sewage sludge.

### 4.2 Residues of veterinary and human medicine

The European Medicine Agency (EMA) is responsible for human and environmental risk assessment of the use of veterinary drugs in Europe. An environmental risk assessment is mandatory for all new applications for veterinary medicinal products, independent of the application procedure (central or national marketing authorisation) (EMA, 2016). If the use of a product results in an unacceptable risk for the environment, then mitigation measures should be proposed by the applicant in order to reduce the risk to an acceptable level. If a risk mitigation measure does not fulfil specific criteria, then the outcome of the risk assessment is that a serious risk for the environment exists. In that case this risk has to be weighed against the favourable aspects of a marketing authorization (EMA, 2016).

When EMA assesses application for specific uses of veterinary drugs, also a risk assessment for the consumer is performed. A toxicological assessment is performed to set acceptable daily intakes (ADI) of each substance in the product. The applicant provides the toxicological data. The ADI is used to set maximum residue levels (MRLs) for different animal products (meat, milk etc.). The withdrawal time (minimum timespan between treatment and slaughtering) is set so the maximum consumer exposure will be below, but often very near, the ADI. The exposure assessment for the consumer is based on the theoretical maximum daily intake, which is the sum of residues present in a standard food basket defined by EMA. This basket is made up of 500 g meat (for mammals 300 g muscle, 100 g liver, 50 g fat and skin, 50 g kidney, and for poultry 300 g muscles, 100 g liver, 10 g kidney and 90 g fat and skin) or 300 g fish plus 1500 g milk, 100 g eggs and 20 g honey (EMEA, 2001). It is assumed that the substance is present at MRL in all the commodities and that the consumer eats the whole food basket every day. That is usually considered a very conservative assumption. Potential human exposure from transfer of veterinary medicinal products via manure or sewage sludge into crops is not considered in the approval process of veterinary medicine. As the exposure from food of animal

origin could be very near ADI, even a minor increase in the exposure from transfer from sewage sludge and animal manures to crops could in theory result in an exceedance of an ADI. As there is almost no investigation on the transfer of veterinary drugs, except antibiotics, into food, also some investigation on the transfer of human medicine has been included in the following. Transfer from manure has also been included. The following should not be considered as a complete review.

The Norwegian Scientific Committee for Food and Environment Safety (VKM) published in 2009 a risk assessment of contaminants in sewage sludge applied on Norwegian soils (Eriksen et al., 2009). Residues of human as well as veterinary medicines were initially considered. For the human and veterinary medicinal products a tiered approach was applied to identify the drug substances that require individual risk assessments.
The starting point of the tiered approach was all 1414 drug substances marketed in Norway.

Tier 0: Initial exclusion of veterinary medicinal products. Exclusion of drug substances due to their properties, i.e. substances not considered toxic (e.g. proteins, vitamins), because of minor use or because of their formulation.

Tier 1: Calculation of maximum PECsludge. Exclusion of drug substances that have a PECsludge lower than the cut-off concentration of 587µg/kg, corresponding to <100µg/kg in soil.

Tier 2: Exclusion of drug substances following a 1st refinement of the PECsludge considering physicochemical properties. Recalculation of PECsludge (Tier 2). Application of the cut-off value as in Tier 1.

Tier 3: Exclusion of drug substances following a 2nd refinement of the PECsludge considering the in vivo drug metabolism in the human body. Recalculation of PECsludge (Tier 3). Application of the cut-off values as in Tier 1 and a cut-off concentration of 59 µg/kg for anticancer drugs and hormones.

Tier 4: Exclusion of drug substances considering experimental data on biodegradation and removal efficiencies in the STPs (Eriksen et al., 2009).

A human risk assessment of the remaining substances was performed. Some medicinal products are used as human medicine as well as veterinary medicine. For these substances VKM use ADIs set by the EMA expert group “Committee for Veterinary medicinal Products for Veterinary use” (CVMP).

For substances without an ADI, VKM used threshold of toxicological concern (TTC) in the human risk assessment. TTC is an approach, which has been used to perform human risk assessment solely based on knowledge of exposure and the chemical structure of a substance (Barlow, 2005). TTC is used when the exposure is very small. The principle is that all chemicals are divided into different groups based on chemical structure. In the original approach, which was used by VKM, there were three groups. Group 1 was substances of simple chemical structure and efficient modes of metabolism, which would suggest a low order of oral toxicity. Group 3 was substances of a chemical structure that permits no strong initial presumption of safety or may even suggest significant toxicity or have reactive functional groups. Group 2 was substances considered to be between group 1 and 3. To determine the highest tolerable human exposure for all substances in each of these groups, toxicological data and health based intake thresholds, like ADI, were collected. These groups consist of substance with well-known toxicity and substance where no or very few toxicological data exist. For each group, the 5$^{th}$ percentile of the ADIs, or other human intake thresholds, were calculated (The 5$^{th}$ percentile is the value where 5% of the ADIs are lower and 95% are higher). These values were used as human intake thresholds for substances of unknown toxicity in each group. The human intake thresholds were calculated to be as follows: Group 1: 30 µg/kg body weight/day, group 2: 9 µg/kg body weight/day and group 3: 1.5, µg/kg body weight/day (Kroes et al., 2004). To allocate a chemical into one of the groups, a decision tree has been developed. This approach can be used for most organic chemicals but there are groups of chemicals, which should not be included. Medicine will usually be in group 3 due to the chemical structure. Initially TTC was meant only to be used for substances of unknown toxicity.

VKM performed an exposure assessment for children to medicine assuming an intake of soil of 0.2 g per day and compared these intakes with the relevant ADIs or TTCs (1.5 µg/kg body weight). Using these principles VKM concluded that: 'The estimated concentrations for all drug substances in soil mixture after use of sewage sludge as soil conditioner is lower than the food safety reference values (TTC, ADI). VKM considers it unlikely that consumption of soil mixture added sewage sludge will pose any risk to the children's health' (Eriksen et al., 2009).

Concerning dietary exposure VKM did not have access or expertise to use models for uptake of drugs in plants after sewage application. Therefore, they were not able to perform a dietary human exposure assessment (Eriksen et al., 2009).

The uptake and effects of a mixture of widely used therapeutic drugs in *Eruca sativa L*. and *Zea mays L*. plants have been assessed (Marsoni et al., 2014). Eight different pharmaceutically active compounds (salbutamol, atenolol, lincomycin, cyclophosphamide, carbamazepine, bezafibrate, ofloxacin and ranitidine) and their presence in the edible parts of the plants were measured. The tested concentrations were the concentrations found in Italian wastewaters and rivers and 10 and 100 times that concentration. Lincomycin and oflaxacin were found above

the limit of quantitation in all conditions tested in *E. sativa*. The results suggest that uptake of some pharmaceuticals from the soil may indeed be a potential transport route to plants and that these environmental pollutants can reach different edible parts of the selected crops. It was also concluded that crops exposed to the selected pharmaceutical mixture would not have any negative effects on human health (Marsoni et al., 2014).

The uptake and translocation of metformin, ciprofloxacin and narasin, which is used in human as well as veterinary medicine, has been investigated in carrot and barley. The root concentration factors (RCF) found was higher than the corresponding leaf concentration factors (LCF) for the three test pharmaceuticals. Ciprofloxacin and narasin showed bioaccumulation factors below 1 for all analysed plant compartments. Metformin showed a generally higher bioaccumulation pattern in roots (RCF 2–10) and leaves (LCF 0.1–1.5). No human risk assessment was performed as the impact on food safety, risk assessment and human health was considered to be beyond the scope of the investigation (Eggen et al., 2011).

Occurrence of 11 typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases in northern China has been investigated. Antibiotics were mainly taken up through water transport and passive absorption in radish, rape celery and coriander. The distribution of antibiotics in the plants was in the sequence leaf > stem > root, and performed biological accumulation (Hu et al., 2010). In general, low concentrations (<10 μg/kg) of the antibiotics were observed, but in coriander higher concentrations (up to more than 500 μg/kg) of some of the antibiotics were found.

In summary, some studies confirm that veterinary medicine may be transferred to crops and therefore be of potential human health concern. There are only very few attempts to perform consumer risk assessment due to the transfer of veterinary medicine to crops, but the risk assessments which has been performed indicates a low risk to the consumer (Eriksen et al., 2009, Marsoni et al., 2014, Eggen et al., 2011, Hu et al., 2010).. The concentration of veterinary medicine in the studies, where no assessment has been performed is low, and it is considered unlikely that they would possess a risk to the consumer.

There are not sufficient studies on the transfer of residues of veterinary drugs in crops due to use of sewage sludge to draw general conclusions concerning consumer safety. The cited literature contains data from animal manure as well as sewage sludge. It is considered likely that the concentration of veterinary medicine is higher in manure than in sewage sludge. Therefore, as there in these investigations has not been identified a human health concern from residues of veterinary medicine in animal manure, it is considered highly unlikely that the presence of veterinary medicine in sewage sludge should be of human health concern due to transfer into crops. The exclusion of veterinary medicine at tier 0 in the Norwegian risk assessment indicates that VKM agrees on this conclusion.

In conclusion, residues of veterinary and human medicine in sewage sludge are considered of low human health concern.

There is not sufficient data to allow a similar conclusion on residues of veterinary medicine in cattle or pig slurry.

### 4.3 Antibiotic resistance in agricultural soils

Antibiotic resistance constitutes a major challenge for public health and the environmental dimensions of antibiotic resistance have lately been widely recognized. This is reflected in the current EU antibiotic resistance action plan emphasizing the so-called One Health approach that acknowledges the need to consider high-risk environmental compartments such as agricultural soils used for food production. Transfer of pathogenic bacteria or of antibiotic resistance from non-pathogenic bacteria in agricultural soils to pathogenic bacteria in humans represent relevant human health risk scenarios (Ashbolt et al., 2013). First of all, soil bacterial communities are known to harbour an extremely diverse collection of antibiotic resistance genes (ARGs) and other resistance determinants such as mobile genetic elements capable of transferring ARGs from non-pathogenic bacteria to pathogenic bacteria (D'Costa et al., 2006; Dantas et al., 2008; Gudeta et al., 2016). Hence, agricultural soils constitute a rich source of novel antibiotic resistance mechanisms yet-to-be recruited by pathogenic bacteria. Secondly, there is now direct evidence that ARG abundance has increased in agricultural soils during the antibiotic era (i.e. since about 1940) (Graham et al., 2016; Knapp et al., 2010) and direct links between bacterial antibiotic resistomes present in agricultural soils and clinical environments have been established (Forsberg et al., 2012; Graham et al., 2016). Several studies have looked into the anthropogenic sources of ARGs in agricultural soils, but no systematic comparative studies of the relative importance of these sources have been carried out. Hence, the available evidence is scattered, but animal manure and sewage sludge are thought to comprise major external sources of ARGs in agricultural soils (Bondarczuk et al., 2016; Heuer et al., 2011; Pepper et al., 2018).

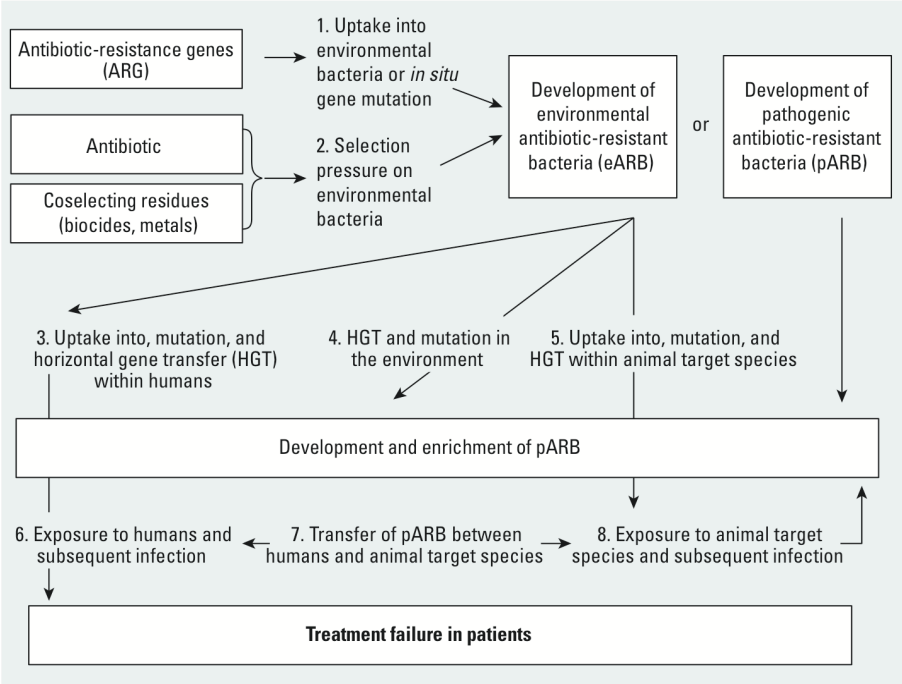


**Figure 4.1**. Conceptual framework for human health risk assessment of the environmental development and transfer of antibiotic resistance (Ashbolt et al., 2013; *Environmental Health Perspectives* 121: 993-1001; reproduced with permission).

A conceptual framework for the environmental processes involved in the environmental development and transfer of antibiotic resistance has been developed (Figure 4.1), but unfortunately, it is not yet possible to perform a human health risk assessment (HHRA) for agricultural soils receiving different inputs such as animal manure or sewage sludge. For instance, we yet even have to agree on a common definition of antibiotic resistance in an environmental context and there is a total lack of standardized procedures for determination of minimal selective concentrations for antibiotic resistance and for estimating the abundance and fates of ARGs (Figure 4.1). Hence, immense knowledge gaps prevent us from reliably quantifying human health risks associated with ARGs in any environmental compartment (Ashbolt et al., 2013; Berendonk et al., 2015; Larsson et al., 2018).

For the purpose of this report we have therefore not been able to perform a quantitative risk assessment of the antibiotic resistance issue and we therefore adopted an expert opinion-driven comparative approach in which we aimed to discuss risks posed by sewage sludge deposition to agricultural land by comparing the risks posed by sewage sludge and manure following their amendment to agricultural soils as based on a systematic literature study. Risk was operationally defined as the ability of sludge and manure to increase abundance of ARGs and their genetic transfer potentials in agricultural soils receiving these fecal inputs in two dimensions (i.e. magnitude and duration of effect) regardless of the underlying mechanisms. Known mechanisms include environmental selection of ARGs caused by selective agents present in sludge/manure (i.e. antibiotic residues), environmental selection of ARGs caused by co-selecting agents present in sludge/manure (i.e other antimicrobials, metals, and biocides), simple deposition of ARGs present in sludge/manure and even ecological disturbance of soil bacterial communities following application of organic fertilizers (Berendonk et al., 2015; Brandt et al., 2015; Udikovic-Kolic et al., 2014).

### 4.3.1 Literature survey

On July 31, 2018, the ISI Web of Science All Databases were searched using three search strings:

1. ((antibiotic resistance or antimicrobial resistance) and soil* and manure*) = 767 hits
2. ((antibiotic resistance gene* or antimicrobial resistance gene*) and soil* and (biosolid* or sewage sludge*)) = 437 hits
3. Search string 1 and 2 combined = 115 hits

A total of 1089 outputs (mainly original papers, but also reviews and other scientific outputs) were retrieved by this procedure and scientific studies fulfilling one of the following criteria were selected for inclusion into this meta study:

1. Relevant studies investigating effects of Danish animal manure or Danish sewage sludge on the prevalence of antibiotic resistance in Danish agricultural soils were included.
2. Relevant studies investigating effects of both pig manure and sewage sludge on the prevalence of antibiotic resistance in agricultural soils using the same methodology and/or soil conditions.
3. Selected studies investigating effects of sewage sludge on the prevalence of antibiotic resistance in agricultural soils from northern Europe and the temperate zone of North America. Studies were selected based on an expert judgement of their relevance.
4. Selected studies on possible ARG mitigation technologies for sewage sludge were selected based on an expert judgement of their relevance.
5. Selected studies not retrieved by the above search strategy were further selected based on an expert judgement of their relevance (e.g. papers on other environmental sources of ARG exposure in humans such as aquatic exposure and exposure during international travel).
6. A series of peer-reviewed review papers dealing with the effects of either sewage sludge or animal manure amendments to agricultural soils on the fate of ARGs and antibiotic resistant bacteria were also consulted (Bondarczuk et al., 2016; Durso and Schmidt, 2017; Heuer et al., 2011; McLain et al., 2017; Pepper et al., 2018; Rizzo et al., 2013; Williams-Nguyen et al., 2016).

### 4.3.2 Initial considerations

Based on the scientific literature, it is well established that even natural, 'pristine' soils harbour a diverse reservoir of ARGs (the soil bacterial antibiotic resistome) (Cytryn, 2013; D'Costa et al., 2011). Most classes of antibiotics are produced by certain soil bacteria and antibiotics have been an integral component of the soil ecosystem for millions of years. Clearly, the presence of the ARGs in natural soils represents a risk to human health and many microbiologists now believe that many, if not most, ARGs in pathogenic bacteria have an environmental origin. However, the risk of ARG transfer from environmental bacteria to pathogenic bacteria in humans depends tremendously on the genomic, species and ecological contexts of the resistance gene (Martínez et al., 2015). Hence, some mobile genetic elements (MGEs) often recruit ARGs and are thought to play a major role for environmental dissemination of ARGs (Gillings, 2013; Gillings, 2018; Gillings et al., 2015; Ma et al., 2017a). Some MGEs such as class 1 integrons are abundant in sewage sludge and manures and thus constitute a risk factor for enhanced mobility of ARGs relative to background ARG prevalence (Gaze et al., 2011). This knowledge is important when evaluating risks posed by sewage sludge and other organic fertilizers. We need to ask the following questions: 'Does sewage sludge application to agricultural land lead to an expansion of the soil bacterial antibiotic resistome (i.e. increased abundance and diversity of ARGs relative to natural background) and does sludge application lead to an increased ARG transfer potential to pathogenic bacteria via enrichment of mobile genetic elements?

### 4.3.3 Sewage sludge and manure as sources of ARGs in Danish agricultural soils

Studies exploring these questions in a Danish context are rare. To the best of our knowledge only one Danish study has directly compared the effects of sewage sludge and manure application on antibiotic resistance in agricultural soil (Riber et al., 2014). This field study took advantage of the long-term CRUCIAL field trial in Taastrup (Magid et al., 2006) and used culturable *Pseudomonas spp* as indicator bacteria. Organic fertilizer amendments corresponding to more than 100 years of application were found to only transiently affect the antibiotic resistance profiles and levels of resistance declined to unfertilized control background levels 9 weeks after application of organic fertilizers. Consistent with these results, other results from the same field trial has documented no or only very minor effects of accelerated rates of sewage sludge application on soil bacterial community composition and Cu resistance relative to unfertilized or NPK fertilized controls (Lekfeldt et al., 2014; Poulsen et al., 2013; Riber et al., 2014). However, another study from the CRUCIAL site indicated an increased permissiveness for plasmid uptake among bacteria from soil fertilized with manure as compared to unfertilized controls, but unfortunately sewage sludge was not evaluated in this study (Musovic et al., 2014). This study thus suggests a higher risk for horizontal gene transfer of ARGs in manured soil, whereas effects of Danish sewage sludge are presently unknown.

Other Danish studies have focused on effects of animal manures on antibiotic resistance in agricultural soils. Using a bacterial cultivation based approach, Sengeløv and co-workers were among the first to demonstrate that levels of antibiotic resistance (resistant relative to total colony forming units) increased in farmland soil following manure application, but also that ARG levels quickly decreased to levels similar to unfertilized controls (Sengeløv et al., 2003). A transient increase of tetracycline resistance was also reported in a microcosm study focusing on enterococci (Agersø et al., 2006). A range of other early Danish studies focused on antibiotic resistance and its genetic transfer potential in bacteria such as *Streptococcus*, *Bacillus*, *Pseudomonas*, *Alcaligenes* and *Arthrobacter* isolated from manured soils (Agersø and Sandvang, 2005; Jensen et al., 2001; Jensen et al., 2002). Collectively, these studies provided early evidence for some of the potential risks for environmental dissemination of antibiotic resistant bacteria and ARGs in agricultural soils receiving animal manures. More recently, Bech and co-workers studied factors influencing the survival and leaching of tetracycline resistant *Escherichia coli* bacteria in two manured soils prone to rapid preferential flow through soil macropores (Bech et al., 2014). Rapid population decline was indicated for both soils (plough layer) and significant leaching of *E. coli* was only observed in one soil (3-130 CFU ml-1). Another recent study indicated a risk for transfer of *E. coli* bacteria from manure to lettuce although other source of bacterial contamination (i.e. 'surrounding environment and wildlife') was also indicated (Jensen et al., 2013). Even more recently, Graham and co-workers quantified

four broad-spectrum β-lactamase ARGs and Class 1 integron genes in archived soils sampled between 1923 and 2010 from a long-term fertilizer experiment comparing effects of manure with inorganic fertilizers in Askov, Jutland (Graham et al., 2016). A number of interesting conclusions were reached from this study. ARG and Class 1 integrase gene abundances (normalized relative to 16S rRNA gene numbers; a gene present in all bacteria) were significantly higher in post-1940 soils from the manure fertilized soils as compared to soils fertilized with inorganic fertilizer. This effect was especially marked for the integrase genes which showed a continuous increase over time indicating an increased potential for genetic transfer of ARGs and other resistance genes known to be often recruited by class 1 integrons (Gillings, 2017). There were also some indications of ARG declines in recent years following the ban of antibiotic growth promoters in Danish agriculture suggesting a role of antibiotic stewardship for reducing environmental loads of ARGs in manured soil. Interestingly, dominant ARGs varied over time in a pattern that roughly paralleled the appearance of the same ARGs in human pathogenic bacteria suggesting a link between the soil and clinical antibiotic resistome.

### 4.3.4 Sewage sludge as sources of ARGs in agricultural soils – evidence from other countries

The most comprehensive field experimental trials investigating effects of sewage sludge application to farmland on the levels of antibiotic resistance in harvested crops have been performed by Ed Topp's research group in southern Ontario, Canada (Lau et al., 2017; Rahube et al., 2016; Rahube et al., 2014). The first of these studies was carried out over two consecutive crop growth seasons (lettuce, carrots, radish, and tomatoes) with application of either untreated sewage sludge or treated sewage sludge sanitized according to local regulations for sludge application to agricultural farmland (Rahube et al., 2014). At no time did sludge treatments result in higher numbers of culturable enteric bacteria on harvested crops than observed for corresponding NPK fertilized control treatments. Further, there were no consistently significant effects of sewage sludge application on the abundance of antibiotic resistant coliform bacteria in soil or on harvested crops. Several ARGs initially could be detected only in sludge-amended soils and their abundance was studied in more detail. Overall, the results suggested that both types of studied sewage sludge had the potential to increase the abundance and diversity of ARGs recovered on harvested crops in the season of sludge application and that a 15-month delay between sludge application and crop harvest was needed to attenuate exposure to sludge derived ARGs. The same field experiment was investigated further in a subsequent follow-up study looking at post sludge application dynamics of a higher diversity of ARGs and mobile genetic elements of importance for ARG dissemination (Rahube et al., 2016). Similar results were obtained suggesting that sewage sludge did not lead to elevated human exposure to antibiotic resistant determinants via harvested crops provided that a 1-year delay between sludge application and crop harvest was ensured. Similar results have also been obtained by the same research group using comparable methodologies in similar field experiments with

livestock manures (Marti et al., 2013; Marti et al., 2014) and with sewage sludge and manure subjected to different pretreatments prior to farmland application (Lau et al., 2017; Tien et al., 2017). In a field study performed in England (UK), it was found that the impact of sewage sludge on ARGs in agricultural soil depended strongly on the application mode (Xie et al., 2016).

In a German study Hölzel and co-workers reported a comprehensive comparison of antibiotic resistance levels in three bacterial species (*E. coli*, *Enterococcus faecalis* and *Enterococcus faecium*) isolated from sewage sludge derived from different sewage treatment plants (n = 111) and liquid pig manures derived from different pig farms (n = 305) in Bavaria (Hölzel et al., 2010). For most tested antibiotics the manure-derived strains displayed a higher frequency of resistance. Multidrug resistance was also most frequent in manure-derived strains. The authors also compared their observed levels of antibiotic resistance to data from the DANMAP survey in Denmark and concluded that sewage sludge antibiotic resistance data were comparable to data from healthy people in Denmark. By contrast, antibiotic resistance levels in German pig manure was higher than corresponding resistance levels in healthy Danish pigs.

Very recently (March 2018), Pepper and co-workers reviewed risks for environmental dissemination of antibiotic resistance associated with farmland application of sewage sludge and other municipal wastes (Pepper et al., 2018). It was concluded that [quote] 'while antibiotic resistance levels in soil are increased temporally by land application of wastes, their persistence is not guaranteed and is in fact variable, and often contradictory based on application site'. Although the paper was written from an American perspective, its conclusion is almost certainly also relevant in a Danish context.

It is generally believed that simple deposition of ARGs and other antibiotic resistance determinants is the main mechanism for expansion of the soil bacterial antibiotic resistome in sludge-amended soils. Hence, concentrations of bioavailable antibiotic residues are generally considered too low to select for antibiotic resistance in agricultural soils (Brandt et al., 2015). However, some studies have linked environmentally relevant concentrations of metals to antibiotic resistance (Knapp et al., 2011; Zhao et al., 2019) and indeed metals may in some cases confer stronger selective agents for antibiotic resistance in soil than antibiotic residues do (Song et al., 2017).

### 4.3.5 Dissemination of antibiotic resistance determinants from agriculture to aquatic recipients

Antibiotic resistant bacteria and ARGs are mobile in the environment and there is thus a risk that resistant bacteria and ARGs derived from animal slurries and sewage sludge may leach from agricultural soils to reach ground water or contaminate nearby aquatic recipients via

surface run-off. Zhu and co-workers (Zhu et al. 2017) examined continental scale pollution with antibiotic resistance genes in estuaries that lie between terrestrial/freshwater and marine ecosystems, acting as natural filtering points for pollutants. ARGs in sediments from 18 estuaries over 4,000 km of coastal China were diverse and abundant, with over 200 different resistance genes being detected, 18 of which were found in all 90 sediment samples. The strong correlations of identified resistance genes with known mobile elements, network analyses and partial redundancy analysis all led to the conclusion that human activity is responsible for the abundance and dissemination of these ARGs. Such widespread pollution with xenogenetic elements was deemed to haves environmental, agricultural and medical consequences.

In Europe, a study from UK has demonstrated that 'surfers are at risk of exposure to and colonisation by clinically important antibiotic-resistant *E. coli* in coastal waters', but the relative importance of different environmental source(s) are not known (Leonard et al., 2018). Hence, we cannot currently know to which an extent animal slurries or sewage sludge are involved in transmission to this environment, but treated or untreated sewage waste water from humans will most likely represent a much more important source as compared to sewage sludge. Glæsner and co-workers (Glaesner et al., 2011) examined interactions in mesocosmos between soil texture and placement of dairy slurry application, and showed that injection decreased leaching of all P forms compared with surface application across soil types. Lower leaching losses were attributed to physical retention of particulate P and dissolved organic P, caused by placing slurry away from active flow paths, especially in the fine-textured soil columns, as well as to chemical retention of dissolved inorganic P, caused by better contact between slurry P and soil adsorption sites. In a follow up study (Glaesner et al., 2016) studied bacteria as transporters of phosphorus through the same soils, and found that upon surface application of slurries the leaching of P more than doubled, and the leaching of P from the bacterial biomass increased from less than 2 % to up to 7.9 % of total P leached.
Thus, appropriate practices for land application of organic fertilizers to agricultural land can mitigate human health risks. E.g. incorporation of slurries and sludge will minimize risks for loss to surrounding water bodies. In many regions such regulations are not in place, and in some regions open lagoons that occasionally spill directly into waterbodies may be found (e.g. Asia, Latin America and the US).

### 4.3.6 Mitigation of ARGs in sewage sludge prior to farmland application

Mitigation of ARG dissemination can be obtained by proper pre-treatment of organic fertilizers before their application to agricultural soils (Pruden et al., 2013; Rizzo et al., 2013). This line of research has boomed in recent years and is especially active in China where the environmental challenges associated with dissemination of ARGs are massive (Chen et al., 2016; Zhu et al., 2013; Zhu et al., 2017). Several technologies have been successfully evaluated, but with mixed results (Burch et al., 2017; Liao et al., 2018; Ma et al., 2011; Sharma et al.,

2016; Su et al., 2015). Although most studies report significant declines in ARG abundance, this is not true for all studies and the only treatment shown to be 100 % effective appears to be thermal treatment (450 °C) to form biochar (Zhou et al., 2019). The variability in the obtained results from composting technologies can to a large extent be explained by significant bacterial community shifts during the composting process, but in extreme cases concentrations of antibiotic residues and co-selective agents (e.g. toxic metals) may also be high enough to select for antibiotic resistance in the composted material.

### 4.3.7 Human ARG exposure and relative risks

When evaluating public health risks associated with environmental dissemination of antibiotic resistance following farmland application of sewage sludge or manure it would be prudent to compare these risks to risks associated with other transmission pathways. These include direct human-to-human transmission, animal-to-human transmission, and transmission of antibiotic resistance to humans via other environmental compartments such as water and air. Unfortunately, such comparisons are impossible to make in part because the ultimate sources of ARGs in pathogenic bacteria are very difficult to establish. International travel is also known to represent an important risk factor and many infections with antibiotic resistant bacteria can now be traced back to infections acquired when Scandinavians travel to countries with higher prevalence of antibiotic resistance (DANMAP 2018; Bengtsson-Palme et al., 2015; Petersen et al., 2015). This can probably be explained at least in part by more relaxed environmental regulation (and enforcement of existing regulations) in low- and middle-income countries in Asia and elsewhere for instance in connection to pharmaceutical production of antibiotics (Larsson, 2014), pollution of waterways (Zhu et al., 2017), and agriculture (Zhu et al., 2013). Large-scale surveys of ARGs in drinking water from Asian cities recently revealed higher relative abundance of ARGs in drinking water than in most sediments and soils (Ma et al., 2017b).

### 4.3.8 Conclusions and perspectives for safe application of sewage sludge for agricultural use in Denmark

Although it is not possible to perform a quantitative risk assessment, the available evidence from the literature does not indicate that application of sewage sludge represents a larger risk than the application of animal manure with regard to dissemination of antibiotic resistance on farmland. Due to the strict requirements in the Danish regulations for land disposal of sewage sludge (Slambekendtgørelsen; Juli 2018) we find it unlikely that application of sewage sludge constitute a significant risk for dissemination of antibiotic resistance, but clearly there is a need for more research to fully justify this conclusion. Even if significant risks will eventually be identified, it should be possible to develop ARG mitigation measures prior to farmland application of sewage sludge.

# PART III

# 5 TERRESTRIAL RISK ASSESSMENT

## 5.1 Cumulative risk assessment approach

In many risk assessment procedures, the risk is quantified by a comparison of the predicted environmental concentration (PEC) and the predicted no effect concentration (PNEC) as the ratio of PEC/PNEC. The general practice is to conduct the exposure and effect assessment for one substance at a time. An important question is however, whether this substance-by-substance approach is sufficient to identify risk from exposure to a large and wide range of multiple substances. Several reports have highlighted the importance of understanding the aggregation of risks from multiple stressors and further recent legislation mandates consideration of cumulative risk in risk assessment processes of e.g. pesticides (U.S. Environmental Protection Agency 2003).

In the substance-by-substance risk assessment approach, each chemical is assessed for its effects on a single or several organisms e.g. children for human risk assessment or aquatic species for aquatic risk assessments etc. In the cumulative risk assessment process, instead of the substance being the central leaving point, the exposed organism is the central part of the assessment, for which the aim is to characterize all relevant risk factors.

In the present risk assessment the organism(s) in the centre is soil-living organisms as e.g. plants, microorganisms and invertebrates. During application of fertilizer either as animal slurry or sewage sludge to agricultural soils, several substances are introduced to the soil environment and hence may be a risk factor for the soil-living organisms. By assessing the risk of all known substances in the slurry or sludge, it is possible to evaluate the total risk, to rank substances based on their potential risk, to identify high-risk substances and hence to evaluate the potential risk in a more realistic scenario.

It is acknowledged that also other factors such as temperature, moisture, predatory pressure, starvation etc. can add additional stress to living organisms and/or enhance the stress of chemical stressors. These non-chemical factors are however not included in present assessment.

The present report is to our knowledge the first to perform a cumulative risk assessment of potential harmful substances in slurry and sludge, and is further the first to compare the two types of fertilizer including the chemicals they may contain that can cause toxicity to soil organisms.

## 5.2 Levels in sludge and slurry

### 5.2.1 Identification of substances for environmental risk assessment

The greatest limitation in risk assessments is the lack of knowledge. More accurately, we only measure the concentrations of compounds we expect or fear to find in a certain matrix. Hence, we only assess the risk of that limited number of compounds, which we already know might potentially pose a risk. However, the aim of the present report is not to look for new compounds of potential risk, but to help future decision makers prioritize between compounds known to be present in organic fertilizers.

The identification of relevant reports and papers included in this work has been performed primarily by search in the database of the Danish Environmental Protection Agency, other EPAs, The European Chemicals Agency (ECHA), and by search on the Web of Science. It has been prioritized to use contamination values for samples from Danish sites, however in cases where such have not been available, it is stated in the report.
It has not been within the scope of this report to make an exhaustive review of relevant literature, but rather to gather sufficient information on levels and toxicity of contaminants in sewage sludge and slurry to perform a risk assessment, and further to identify potential problematic chemicals. Compounds where either the information on levels or on effects has been lacking from literature have been excluded from the final cumulative risk assessment.

In 1987 The Danish Nationwide Monitoring and Assessment Program for the Aquatic Environment (NOVA-2003) was established under the Danish EPA. The initial years focus was on nutrient levels and organic material. A revision in 1998 prioritized a surveillance of heavy metals and environmental contaminants. The surveillance included measurements at the point sources, e.g. wastewater treatment plants, leaching from agricultural fields and atmospheric deposition. In 2001-2002 slurry samples were also included in the analysis.
In 2004 the program was expanded under the name The National Program for Surveillance of the Aquatic Environment and Nature (NOVANA). Monitoring of contaminants in sludge was followed under the sub-program Point sources. The latest report on contaminants in sludge was published in 2015 (NOVANA 2015) covering data collected in the period 2004-2009 or 2004-2012 (depending on contaminant group). The resulting reports were used as the basis for levels of contaminants in Danish sludge and slurry. As the focus of the program has shifted throughout the years, both in respect to sampled matrices and measured compounds, the most recent data was chosen for each matrix and compound. Knowing that quality of wastewater treatment as well as public use of certain compounds might have changed since the publication of these data, levels should be evaluated with care.

*Metals and organic contaminants*

The most recent data on metals and organic compounds in Danish sewage sludge is, to our knowledge published in NOVANA (2015). NOVANA (2015) comprises data from an extended surveillance and hence includes analyses of more compounds, than is tested for in the regular quality analysis of sludge (see Table 2.1).

There is no regular monitoring of contaminants in Danish farmyard slurry. However as mentioned above, in 2001-2002 the National Environmental Research Institute in co-operation with five Danish Counties analysed samples of slurry from livestock for a number of heavy metals and organic compounds (Schwærter and Grant 2003). Cu and Zn were further analysed in 2015 by Bak et al. (2015). To our knowledge, these are the most recent reports on the topic, and hence resulting data were used as a basis for metals and organic contaminants in slurry[1].
The amount of copper and zinc, added to animal feed to prevent disease, has been regulated as per February 2019 and in respect to zinc, will be fully phased out by July 2022 (SEGES, 2019). In addition, in 2016 regulations were made to limit the amount of slurry from piglets used as fertilizer, as these contain higher levels of Cu and Zn than slurry from adults. In the present risk characterization, metal levels in slurry and application rates are based on before 2016 regulations. However a risk characterization based on levels expected/predicted (SEGES, 2019) after 2022 is also included.

*Medicines and estrogenic compounds*

Residues from human and veterinary medicines in sewage sludge and slurry are not regularly monitored and literature on the topic is sparse. In 2008 NOVANA conducted a screening project on emerging contaminants in the aquatic environment (Mogensen et al. 2008). This included a screening of 25 pharmaceuticals in sludge, out of which eight were detected. These eight pharmaceuticals have been included in the present assessment. As a follow-up to this, three sludge samples from one waster water treatment plant were analysed for a selected set of pharmaceuticals (Jensen 2012). Of these five were detected and included in the present assessment.
In addition to levels of metals and organic contaminants in slurry, Schwærter & Grant (2003) also reported levels of 6 (out of 8) antibiotics. These are likewise included in the present assessment.

[1] A discussion arose over the values estimated for Cu and Zn excretion based on the study by Bak et al. (2015). It was argued that concentrations of Zn and Cu in some of the sampled slurries were exceedingly high, and it was questioned if this could be due to sampling errors. We recognized that taking representative samples from animal slurry may be very difficult, and that it could therefore be possible that the sampling was unrepresentative and biased towards too high concentrations. Therefore, we have subsequently based our estimates of excretion of Zn and Cu on physiological model data on pigs, considering the highest legal input of Zn and Cu in feed before new restrictive regulations were changed (SEGES, 2019). As a consequence, the excretion of Cu and Zn was moderated relative to prior estimates, but the tendencies were unchanged, and pig slurry is still seen as the most 'toxic' nutrient source in the 100-year scenario.

Levels of estrogenic compounds are not available for Danish slurry or sludge, and hence values were adopted from Norwegian and U.S. studies (Thomas 2007; Raman et al. 2004).

*Recommendations from previous risk assessments*

The initial literature search further identified recently published and relevant risk assessments on selected contaminants in sewage sludge. The results and recommendations of these risk assessments were taken into consideration and hence contaminants identified as potentially harmful to the environment by the authors, and which were not already included, were added to the list of included compounds. Consequently, octylphenol, polychlorinated naphthalenes, polychlorinated alkanes, triclosan and triclocarban were included in the assessment, as recommended by NOVANA (2008), Eriksen (2009) and Jensen (2012) respectively. Information on levels was adopted from Miljøstyrelsen and Mogensen et al. (2004; 2008). To our knowledge concentrations of polychlorinated naphthalenes, polychlorinated alkanes and triclocarban have not been determined in Danish sewage sludge. For these chemicals international values have been used for derivation of PEC (Stevens et al. 2003; Heidler, Sapkota, and Halden 2006).

Parabens were also identified as relevant for future evaluation (Jensen 2012), however due to the vast range of congeners and the concurrent lack of knowledge on concentrations in sewage sludge/slurry and toxicity towards terrestrial organisms, this group of compounds was not included in the present assessment.

The following compounds or compound groups were therefor included:

- Metals
- Chlorophenyls
- Dioxins
- Furans
- Halogenated aliphatic and aromatic hydrocarbons (HAH)
- Linear alkylbenzenesulfonates (LAS)
- Polyaromatic hydrocarbons (PAH)
- Polybrominated diphenyl ethers (PBDE)
- Polychlorinated biphenyls (PCB)
- Poly- and perfluorinated alkylated substances (PFAS)
- Phenols (including octylphenol)
- Phosphate-triesters
- Phthalates
- Polychlorinated naphtalenes (PCN)
- Polychlorinated alkanes, short chained C10-C13 (PCAshort)
- Polychlorinated alkanes, medium chained C14-C17 (PCAmedium)
- Triclosan

- Triclocarban
- Medicines
- Estrogens

### 5.2.2 Concentrations of substances in slurry and sludge

An exhaustive list of included compounds and their respective concentrations in slurry and/or sludge is given in Appendix B, Table 1 PART I-IV.

## 5.3 Exposure assessment

### 5.3.1 Exposure estimation and calculation of predicted soil concentration

Exposure assessment for the soil compartment is important with respect to exposure to terrestrial organisms. In this case fate and distribution of the released compounds in the soil compartments are estimated in order to calculate the predicted environmental concentrations (PEC). These estimated concentrations are used as exposure concentrations.
PEC in soil is calculated as recommended by The European Chemicals Agency (ECHA, 2016) and as elaborated below.

$$PEC_{init} = \left(\frac{D_{air}}{k}\right) + \left(\frac{1}{kT}\right) * \left(C_{init} - \frac{D_{air}}{k}\right) * \left(1 - e^{-kT}\right) \quad \text{Eq. 1}$$

Where $t$ = 30 days and $PEC_{init}$ (mg/kg) the predicted environmental concentration in soil averaged over the 30 days initially after application of slurry or sewage sludge. $C_{init}$ is the concentration in soil initially after sludge or slurry application and takes into account the natural background concentration of certain compounds. In the present assessment, $C_{BKG}$ was set to 0 mg/kg for both organic compounds and metals. For organic compounds this is based on the assumption, that they will not be naturally present in soil. For metals, several of the PNEC values used to evaluate the risk, are referring to added rather than total metal concentration, and hence background levels are discussed where appropriate. $D_{air}$ was set to zero for all compounds, as this input was considered insignificant for the included chemicals. $C_{init}$, the initial concentration in soil after first application of slurry or sludge, was calculated using concentrations identified as described in section 5.2.
The predicted soil concentration after additional applications of slurry or sludge was calculated using Eq. 2.

$$PEC_t = C_{BKG} + \left(\frac{D_{air}}{k}\right) + C_{init} * \left(1 + \sum_{n=1}^{t-1} Facc^n\right) \quad \text{Eq. 2}$$

The total removal rate constant $k$ ($d^{-1}$), is made up of three parts; the biodegradation constant $k_{bio\text{-}soil}$; volatilization of substance from soil $k_{volat;}$ and leaching to deeper soil layers $k_{leach}$. For metals only $k_{leach}$ was taken into account.

$$k = k_{bio-soil} + k_{volat} + k_{leach} \quad \text{Eq. 3}$$

$$k_{bio-soil} = \frac{Ln(2)}{DT50} \quad \text{Eq. 4}$$

$$\frac{1}{k_{volat}} = \left(\frac{1}{kasl_{air} * K_{air-water}} + \frac{1}{kasl_{soil-air} * K_{air-water} + kasl_{soil-water}}\right) * K_{soil-water} * Depth_{soil} \quad \text{Eq. 5}$$

$$k_{leach} = \frac{Finf * preciptation\ rate}{K_{soil-water} * Depth_{soil}} \quad \text{Eq. 6}$$

Facc is the fraction of contaminant remaining in soil to time t, defined as $e^{-(t*k)}$. DT50 is the degradation halftime in soil (d), kasl the partial mass transfer coefficient ($d^{-1}$), $Fx_{soil}$ is the fraction of x in soil, $K_{air\text{-}water}$ and $K_{soil\text{-}water}$ is the air- and soil-water partitioning coefficient respectively ($m^3/m^3$), Finf is the fraction of rain water, that infiltrates into soil. The partitioning coefficients are determined by Eq. 7-8.

$$K_{air-water} = \frac{Henrys\ law\ constant}{gas\ constant * temp\ (K)} \quad \text{Eq. 7}$$

$$K_{soil-water} = Fair_{soil} * K_{air-water} + Fwater_{soil} + Fsolid_{soil} * \frac{Kp_{soil}}{1000} * Density_{solid} \quad \text{Eq. 8}$$

Where $K_p$ is the solid-water partitioning coefficient (L/kg), determined by Eq. 9 using the organic carbon-water partitioning coefficient, $K_{OC}$ (L/kg).

$$K_P = F_{OCsoil} * K_{OC} \quad \text{Eq. 9}$$

Values for constants are listed in Table 5.1.

The recommended method for estimation of PEC (ECHA, 2016) is common practice within risk assessment. The method is however theoretical and generic and hence the resulting estimates should be considered with care as these are subject to large unavoidable uncertainties.

For further explanations and derivations of equations and constants, the reader is referred to the original guidance document (ECHA, 2016).

PEC is estimated individually for application of sewage sludge, farmyard slurry from cattle and slurry from pigs. Application rates are set to match the maximum allowance for P, in order to simulate worst-case scenarios. PEC is estimated initially after first application and additionally after 10 and 100 years, corresponding to 10 and 100 applications for slurry and yearly sludge application and 3 and 33 applications for sewage sludge applied every 3rd year. Even though the average amount of sludge applied per year is the same in the two scenarios, the amount of sludge applied per application determines the contaminant concentration in the soil initially after application. If contaminant concentrations reach a critically high level in the soil initially

after application, this might have effects on soil living organisms that may (partly) persist even after contaminants are degraded. The scenario with application of sludge amounts corresponding to 90 kg P/3rd year reflects Danish practice for sewage sludge application.

**Table 5.1**. Constants used for estimation of PEC. Other constants are explained in the text.
[a] Bulk density of average Danish agricultural soil, [b] suggested by The European Chemicals Bureau (2003), [c] max application rate of P as directed by (Miljø- og Fødevareministeriet 2017b). As P application rate differs for fattening pigs (39 kg P/ha/year) and pigs with piglets (35 kg P/ha/year), the average was used.

| Constant | Value |
|---|---|
| Density of soil | 1.5 kg/L[a] |
| Density of solids | 2.5 kg/L[b] |
| Depth soil | 0.2 m[b] |
| $Fair_{soil}$, $Fwater_{soil}$, $Fsolid_{soil}$ | 0.2, 0.2, 0.6[b] |
| $F_{OCsoil}$ | 0.02 $kg_{OC}/kg_{soil}$[b] |
| $kasl_{air}$ | 120 m/d[b] |
| $kasl_{soil\text{-}air}$ | 0.48 m/d[b] |
| $kasl_{soil\text{-}water}$ | $4.8*10^{-5}$ m/d[b] |
| Finf | 0.25[b] |
| Precipitation | $1.92 \cdot 10^{-3}$ m/d[b] |
| Gas constant | 8314 $Pa \cdot m^3/mole \cdot K$ |
| Temp | 5 ºC |
| **Application rates** | |
| Sewage sludge, 30 kg | 30 kg P/ha/year[c] |
| Sewage sludge, 90 kg | 90 kg P/ha/3year[c] |
| Cattle slurry | 30 kg P/ha/year[c] |
| Pig slurry | 37 kg/P/ha/year[c] |

### 5.3.2 Physical-chemical properties of included compounds

For each of the included compounds physical-chemical properties such as partitioning coefficients for octanol-water ($K_{OW}$) and soil organic carbon-water ($K_{OC}$), half-lives etc. are adopted from the Danish QSAR database (National Food Institute 2018). A reference number to the individual chemical datasheets are given in Appendix C, Table 2 PART II and III. Experimentally derived values are preferred if available in the database. A Henrys law constant was not available for a range of the included PFASs and the QSAR estimated constant was evaluated to be unrealistically high (in the order of $10^4$ - $2*10^6$ Pa $m^3$/mole). Henrys law constant for these was set to value an arbitrary, but low and conservative value of 0.5 Pa $m^3$/mole, to ensure that evaporation from soil was not overestimated. For other substances where a QSAR report is not available, data was adopted from alternative sources. Metals are

not included in the QSAR estimation tool, and properties were adopted from literature. Used references are available from Appendix C, Table 2 PART I-III.

### 5.3.3 Predicted environmental concentrations (PECs)

The estimated soil concentrations of included compounds initially after 1st, 10 and 100 years of soil amendment with slurry and sludge are summarized in Appendix C, Table 3 PART I-III.

PECs are primarily estimated based on reported mean values. If only a range of concentrations is reported in the original reference, the max concentration is used for PEC estimation. When relevant this is indicated in aforementioned Tables in Appendix B and included in the individual risk characterizations. Likewise is the use of slurry or sludge concentrations of non-Danish origin.

## 5.4 Effect assessment

### 5.4.1 Prioritization in derivation of predicted no effect concentration

The strategy used for establishing predicted no effect concentrations (PNECs) is based on the recommendations by the European Chemical Agency (2008). The PNEC is established on the basis of the quality and quantity of the available ecotoxicological information and the use of corresponding assessment or safety factors (see Table 5.2). Hence, if only short term toxicity data of a chemical is available (for one or several species), the lowest available L(E)C50 value is divided by an assessment factor of 1000. If, on the other hand, toxicity data is vast (e.g. species sensitivity distributions) a low assessment factor of 1-5 may be used. Additional information on the use of SSD and associated AF can be found in ECHA (2008).
For existing substances toxicity data for terrestrial organisms is scarce, and hence his report adopted a ”tiered approach” to derivation of PNEC values. PNEC values derived by other reports by thorough assessment of available knowledge of sufficient quality were used as a first priority value. When no PNEC value for soil was available, such was estimated from the aquatic PNEC using equation 10 (ECHA, 2008). If neither soil nor aquatic PNEC were available, a PNEC value was estimated from QSAR predicted toxicological endpoints, and an appropriate assessment factor was applied.

Hence, the method to derive PNEC was prioritized as follows:
1. $PNEC_{soil}$ values derived by other scientific reports
2. Calculated from $PNEC_{aq}$ derived by other scientific reports, using equation 10
3. Calculated from QSAR estimated endpoint (E/LC50) using equation 10 and an assessment factor of 1000

$$PNEC_{soil} = \frac{K_{soil-water}}{soil\ density} * PNEC_{aq} * 1000 \quad \text{Eq. 10}$$

**Table 5.2**. Assessment factors for derivation of $PNEC_{soil}$ (modified from ECHA, 2008).

| Information available | Assessment factor |
|---|---|
| L(E)C50 short-term toxicity test(s) (e.g. plants, earthworms, or microorganisms) | 1000 |
| NOEC for one long-term toxicity test (e.g. plants) | 100 |
| NOEC for additional long-term toxicity tests of two trophic levels | 50 |
| NOEC for additional long-term toxicity tests for three species of three trophic levels | 10 |
| Species sensitivity distribution (SSD method) | 5-1, to be fully justified on a case-by case basis |
| Field data/data of model ecosystems | case-by-case |

When estimating $PNEC_{soil}$ from $PNEC_{aq}$, it is further recommended that the resulting PEC/PNEC value for compounds with Kow > 5, is increased with an additional factor of 10, to take into account the possibility of direct ingestion of soil-bound compounds (ECHA, 2008).

### 5.4.2 Predicted no effect concentrations (PNECs)

PNEC values have generally been derived as described above. However, for some compounds adequate toxicity information has not been available for PNEC estimation. For these compounds alternative methods have been used. Even though resulting toxicity values are hampered with uncertainty, inclusion of these compounds is considered a conservative approach to the cumulative risk assessment. Whenever an alternative approach is used, it is noted in Appendix D, Table 3 PART I-III, and further briefly described below and discussed in the risk characterization where appropriate.

In summary, toxicity information is lacking for several congeners belonging to the group of polybrominated diphenyl ethers. For congeners where data is unavailable, toxicity information on a like congener is used instead. Nineteen unspecified PAHs have been analyzed in slurry, the concentration of these are however only available as $\sum PAH_{19}$. In order to estimate PEC and PNEC value for $\sum PAH_{19}$ in slurry, the mean value of each physical-chemical constant and toxicity data for the 21 single PAHs, for which data are available, are used. An additional assessment factor of 10 was applied to the resulting PNEC.

In respect to dioxins and furans, PNECs has been estimated using an alternative approach than described in section 5.4.1. Dioxins and furans refer to broad classes of compounds that resemble each other in chemical structure and in toxic effects. To describe the toxicity of the different congeners the concept of toxic equivalency factor (TEF) has been developed (Van den Berg et al. 2006). In short, toxicity of the individual congeners is given relative to the most toxic dioxin

(TCDD), and described by respective TEF values. Hence, a TEF value of 0.1 is equivalent to a toxicity of 1/10 of that of TCDD. TEFs are adopted from the US EPA on human risk assessment (US EPA 2010).

Resulting PNEC values are summarized in Appendix D, Table 3 PART I-III along with information of the toxicity data and assessment factor used for deriving the PNEC.

## 5.5 Risk characterisation

The present risk assessment aims at characterizing the potential risk for soil-living organisms when applying animal slurry and sewage sludge to agricultural soils. The potential risk is calculated by comparison of derived values for exposure (section 5.3) and effects (section 5.4).

### 5.5.1 Knowledge gaps

Several factors are determining for the concentration compounds will reach in the environment. In this assessment the majority of these factors have been estimated using QSAR prediction tools. Adopting QSAR estimates introduce uncertainties in the estimated soil concentrations, but allows for inclusion of a wide range of compounds, that would otherwise have been excluded from the risk assessment, both due to time limitations and lack of experimental information.

Disappearance of chemical substances from soil is estimated not taking plant uptake into account. For some chemicals, removal by plant uptake (especially water soluble compounds) can however play an important role in determining soil concentrations. Generally, the mean concentration of the compound in slurry or sludge is used for estimation of PEC. However, in some cases only a concentration range is available, and PEC is estimated based on maximum values. Additionally, some compounds are observed in less than 100 % of the analyzed samples, and the mean value hence calculated based on the samples in which the compounds were observed. This is the case for several of the medicines found in sludge and slurry respectively, and might therefore result in an overestimation of resulting soil concentrations, as we assume they are present in all slurry or sludge samples.

Further, for a few compounds levels in Danish slurry or sludge are not available and hence PECs are calculated based on international values. This is the case for PCN, PCA, triclocarban and estrogens. As mentioned previously, the method used for estimating PEC values are theoretical and generic, and hence resulting PEC values should be regarded as an estimate with large uncertainties.

Of the included compound groups, soil toxicity of metals is best described. Several of the $PNEC_{soil}$ values for metals are derived from species sensitivity distributions and are considered

of high quality. It should however be kept in mind, that metals can exist as different forms/species depending on soil conditions, and hence also toxicity can vary.
There is in general a great lack of toxicity information on organic chemical substances in soil. The majority of $PNEC_{soil}$ values used in the present assessment are derived from $PNEC_{aq}$, which for some groups of compounds have been shown to be a good estimate. However, to what extent this conversion is valid is unknown. For a few compound groups (PBDE, PFAS and phthalates) the toxicity of more than half of the included congeners are estimated from QSAR predicted aquatic toxicity, which is fraught with uncertainties. These PNEC values should be evaluated with great care.

Finally, it should be noted, that the assessment is based solely on the *investigated* compounds. Compounds not included in the present assessment could be present in sludge increasing the toxicity.

### 5.5.2 Cumulated Risk

As explained in section 5.1 the cumulative risk is calculated as the sum of PEC/PNEC for all included substances. A PEC/PNEC > 1 indicates a potential risk for soil-living organisms. The risk level is divided into high risk for compounds with PEC/PNEC >1, medium risk for PEC/PNEC between 0.1 and 1, and low risk for compounds with PEC/PNEC below 0.1.

Figure 5.1A shows the cumulated PEC/PNEC initially after 1, 10 and 100 years of application of cattle and pig slurry and sludge. For slurry, both cattle and pig, application is performed once per year. For sewage sludge two application scenarios are included: application corresponding to 30 kg P/ha/year and corresponding to 90 kg P/ha/3 year. PEC/PNEC values are further summarized in Appendix E, Table 4 PART I-III.

The estimations predicted that the cumulated PEC would reach or exceed the cumulated PNEC already after the first application of fertilizer. Application of slurry from cattle and pig resulted in an initial ∑PEC/PNEC of 0.77 and 0.79 whereas application of sewage sludge in the two scenarios resulted in ∑PEC/PNEC of 3.06 and 9.19. Assessing ∑PEC/PNEC after 100 years of repeated application, slurry from cattle and pig resulted in a ∑PEC/PNEC of 2.06 and 8.83 respectively, whereas application of sewage sludge in the two scenarios resulted in ∑PEC/PNEC of 4.89 and 10.78. The difference between ∑PEC/PNEC initially and after 100 years, was markedly larger for slurry fertilizers than for sludge. Slurry fertilizers contain higher concentrations of metal compounds that are not easily removed from the soil, and hence tend to accumulate over time, increasing soil PEC. It should however be noted that in the present risk assessment uptake and removal of metal compounds by harvested plants have not been taken into account.

Generally the ∑PEC/PNEC > 1 is indicating, that there might be a potential risk of adverse effect towards soil-living organisms as result of application of these fertilizers. The calculated risk refers to the month initially after fertilizer application, and hence to the point in time where soil contaminant levels is at their maximum.

To assess the potential long-term exposure to contaminants from slurry or sludge, PEC values in soil six months after application in the 100$^{th}$ year were calculated. After six months the ∑PEC/PNEC of slurry from cattle and pig was 1.42 and 8.23 respectively. Metals accounted for more than 90 % of the summed risk. In respect to sludge the ∑PEC/PNEC after six months were estimated to 2.22 and 3.10 respectively. In these scenarios metals accounted for 72 % and 52 % of the summed risk. These results are illustrated in Figure 5.1B. Results show that the summed risk of the organic compounds is markedly decreased six months after application.

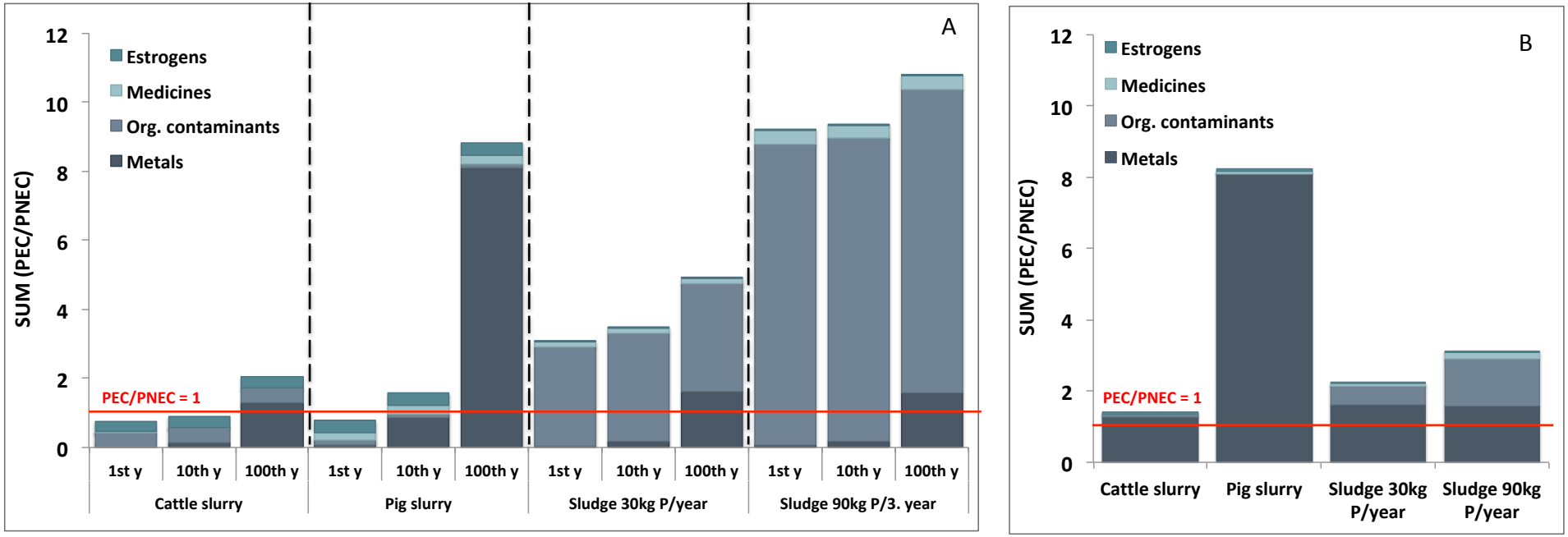


**Figure 5.1.** Calculated ∑PEC/PNEC values for the four major compound groups included. **A.** Year 1, 10 and 100 refers to the 30 days initially after 1st., 10th and 100th year of application. **B.** ∑PEC/PNEC values 6 months after application in the 100th year.

### 5.5.3 Risk characterization of metals and inorganic compounds

Twenty metals are included in the present risk assessment for their potential risk towards soil-living organisms. Of these only five have been determined in slurry. A comparison between the risk associated with the use of slurry and sludge as fertilizers is performed where appropriate.

Resulting PEC/PNEC values of individual metals are summarized in Appendix E, Table 4 PART I and the cumulative risk of metals is illustrated in Figure 5.2 and 5.3 for cattle slurry and sewage sludge, and pig slurry respectively.

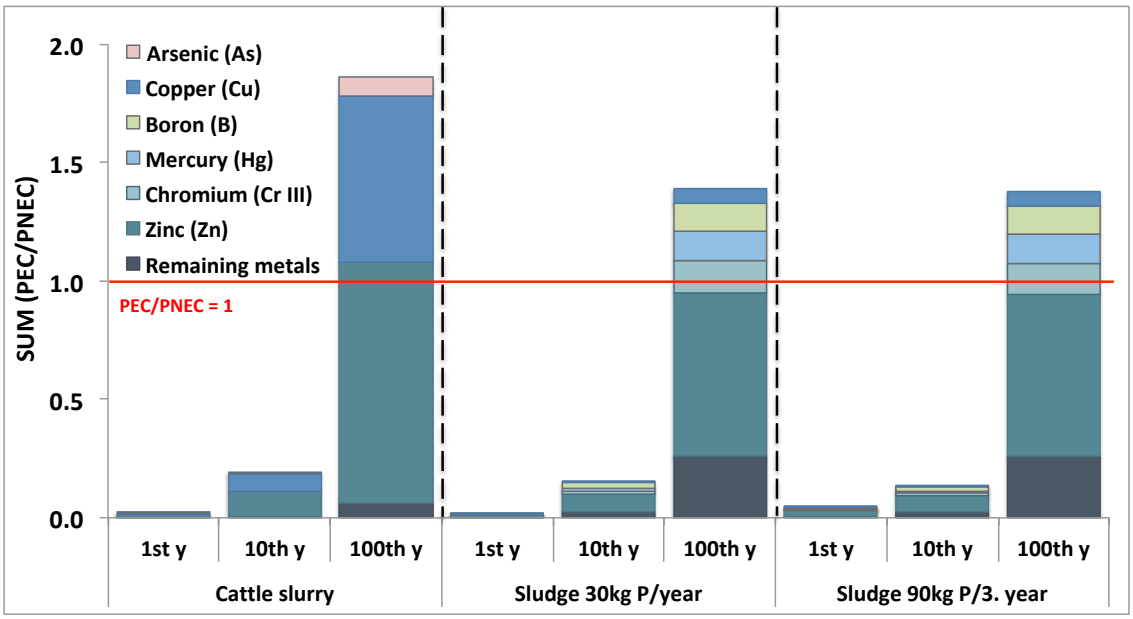


**Figure 5.2.** Calculated ∑PEC/PNEC values for metals with PEC/PNEC ≥ 0.1. Metals with PEC/PNEC below 0.1 are summed as PEC/PNEC of remaining metals. Year 1, 10 and 100 refers to the 30 days initially after 1st., 10th and 100th year of application. Due to the large differences in ∑PEC/PNEC, Pig slurry is shown in Figure 5.3.

The ∑PEC/PNEC of metals after 1, 10 and 100 years is 0.01, 0.14 and 1.28 for application of cattle slurry, 0.09, 0.86 and 8.11 for application of pig slurry. For sludge the resulting ∑PEC/PNEC is 0.02, 0.18 and 1.61 for 30 kg P/year sludge application, and 0.06, 0.17 and 1.60 for 90 kg P/3$^{rd}$ year sludge application.

The main metals contributing to the toxicity in slurry is zinc and copper, accounting for more than 50 % and 90 % of the summed risk in cattle and pig slurry respectively. Zinc is the only metal reaching a PEC higher than its PNEC over a 100-year period on a substance-by-substance approach.

In sludge the main metals contributing to the toxicity are zinc > arsenic > chromium > mercury, jointly accounting for approximately 70% of the summed risk of metals. No single metal is estimated to reach a PEC larger than its PNEC during a 100-year period of sewage sludge application.

Zinc and copper are used as additives in animal feed and medicines, especially for young piglets, resulting in high concentrations of these metals in slurry from piglets. To minimize the amount of Cu and Zn introduced to the soil, in 2016 restrictions were made that limited the application of slurry coming solely from piglets to 14 kg P/ha (from 35 kg P/ha). In 2019 the maximum allowed concentration of Zn in feed was reduced to 2300 mg/kg food. Finally, additional regulations are expected to be enforced July 2022, prohibiting application of Zn to food and further reducing the amount of Cu (SEGES, 2019). The expected PEC/PNEC values for zinc and copper in agricultural soils after application of pig slurry are shown in Figure 5.3 using both before 2016 and after 2022 regulations.

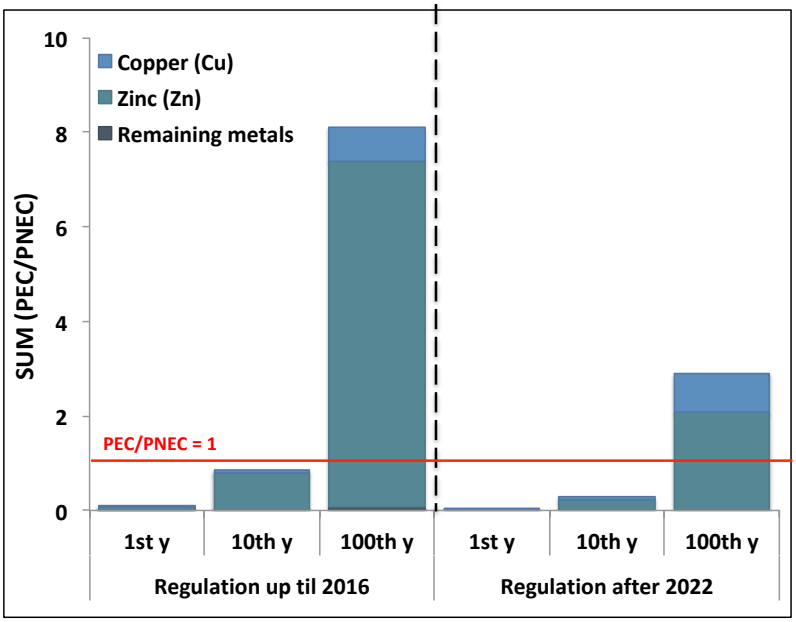


**Figure 5.3.** Calculated ∑PEC/PNEC values for metals with PEC/PNEC ≥ 0.1 for pig slurry. Metals with PEC/PNEC below 0.1 are summed as PEC/PNEC of remaining metals. Year 1, 10 and 100 refers to the 30 days initially after 1st., 10th and 100th year of application. PEC/PNEC is estimated based both on the regulation prior to 2016 and after 2022 of Zn and Cu content in pig feed.

The resulting ∑PEC/PNEC > 1 indicates that metals may reach soil concentrations after application of either slurry or sludge that may cause adverse effect to soil organisms. The risk is approximately 6 times higher for application of pig slurry than for sludge and cattle slurry.

When assessing the risk of metals in soils, it should be taken into account, that metals are chemicals of natural origin and will occur naturally in soil environments. Hence for some metals the expected increase in concentration due to application of fertilizer may be more important, than the actual concentration. Further, the speciation of the metal, adsorption and bioavailability is of paramount importance, but will depend highly on the environmental conditions.

The ECHA model (ECHA, 2016) does not deal with a wealth of these factors that effectively diminishes the toxic effects of substances. Especially removal by plant uptake and leaching may have a substantial effect over time (Smolders, 2013), as well as ageing and the effects of soil pH (Brokbartold et al., 2012; Lock and Janssen, 2003) that will decrease the bioavailability as discussed in detail in the section on heavy metals and human risk assessment.

The present assessment is not meant as an exhaustive evaluation of the toxicity of metals, the aim is however to identify potential problem metals and to compare the expected impact of soil amendment with slurry and sewage sludge. Factors listed above will be discussed for selected metals when considered necessary and where sufficient information is available.

In Table 5.3 available information on natural background concentrations of included metals are listed and compared with the highest calculated PECinit in the present assessment (from Appendix C, Table 3).

**Table 5.3.** Summary of natural soil concentrations of selected metals and estimated maximum increase of soil concentration per year as result of fertilizer application (calculated using the fertilizer type with the highest PEC). All listed concentrations are in mg/kg dw.

| Compound name | Natural soil conc. Range and (typical value) | Max. increase per year due to fertilizer application |
|---|---|---|
| Antimony (Sb) | | |
| Aluminium (Al) | | |
| Arsenic (As) | 0.1-50 (5)[a] | 0.03% |
| Barium (Ba) | | |
| Lead (Pb) | 5-100 (20)[a] | 0.05% |
| Boron (B) | 2-270[b] | |
| Cadmium (Cd) | 0.003-0.9 (0.2)[a] | 0.15% |
| Copper (Cu) | 2-60 (10)[a] | 5.00% |
| Cobalt (Co) | | |
| Chromium (Cr) | 1-100 (15)[a] | 0.50% |
| Mercury (Hg) | 0.01-0.4 (0.08)[a] | 0.28% |
| Molybdenum (Mo) | | |
| Nickel (Ni) | 0.1-50 (7)[a] | 0.11% |
| Selenium (Se) | | |
| Silver (Ag) | | |
| Thallium (Tl) | | |
| Tin (Sn) | | |
| Uranium (U) | | |
| Vanadium (V) | | |
| Zinc (Zn) | 10-100 (30)[a] | 7.15% |

[a](Kjeldsen and Christensen 1996), [b](Kjølholt et al. 2002)

Characterizations of selected metals are elaborated below. Focus has been on metals with medium and high-risk potential (PEC/PNEC ≥ 0.1 based on 100 years of application), i.e. Zn and Cu, As, B, Cr and Hg. This also covers the metals for which natural background concentrations in soil may be increased more than 50% over 100 years as a result of fertilizer application (Cr, Cu and Zn). Remaining metals are characterized less thoroughly.

#### *5.5.3.1 Zinc (Zn) and copper (Cu)*

Zink and copper are the metals generally receiving the greatest attention in respect to animal slurry. Both metals are used as additives in animal feed and medicines, and in accordance, results of the present assessment shows that zinc and copper are the metals reaching the highest PEC values after slurry application. PEC of zinc and copper in soil after 100 years of application with pig slurry is 200 and 48 mg/kg respectively. For comparison, estimated concentrations in soil after cattle slurry or sludge applications are 26 and 18 mg/kg for zinc and 8 and 0.7 mg/kg for copper. The natural background of zinc and copper in Danish soils is 10-100 and 2-60 mg/kg, with typical values of 30 and 10 mg/kg (see Table 5.3), and the use of pig slurry is estimated to increase natural background concentrations of zinc and copper with approximately 7 and 5 % per year (see Table 5.3). This is in accordance with a recent report on zinc and copper in soil after application of slurry concluded that agricultural use of pig slurry has led to a significant increase in soil concentrations of both zinc and copper, which is in accordance with the results of the present assessment (Jensen et al. 2016)

PNEC values for zinc and copper are based on species sensitivity distributions and are considered well founded. Cu is generally less toxic than zinc (see Appendix D, Table 3, PART I), and is present in lower concentrations.

Results of the risk assessment shows, that zinc reaches PEC values close to or above its PNEC after an application period of 10 and 100 years for pig and cattle slurry respectively, whereas PEC values are below PNEC even after 100 years of sludge application. The respective PEC/PNEC values after 100 years are 1.02, 7.34 and 0.69 for cattle slurry, pig slurry and sludge.

For copper, the resulting PEC/PNEC values after a 100 years of fertilizer application are 0.12, 0.71 and 0.01 for cattle slurry, pig slurry and sludge respectively (see Appendix E, Table 4, PART I).

A national monitoring program for heavy metals in soil, was initiated in 1990 by the Danish EPA. (Bak et al, 1997). It was at first concluded that heavy metals found in arable soil and on nature areas did not give rise to concern. However, in a later round of the monitoring program, Jensen et al (2016) found that amendment of soils with pig slurry had led to a significant increase in soil concentrations of copper and zinc, especially in the latest monitoring period from 1998 to 2014. Thus, predicted no-

effect concentrations for soil dwelling species published by the European Union was exceeded for zinc in 45% of all soil samples, with the highest proportion on sandy soils. This agrees well with our predictive modeling.

A risk assessment by the Norwegian Scientific Committee for Food Safety (Eriksen et al. 2009), evaluating the adverse effects on soil-living organisms after application of sewage sludge, concludes that zinc may pose a risk on sandy soils (PEC/PNEC of 2-3), but is less likely to pose a risk when applied to clay-like soils (PEC/PNEC of 0.2-0.7). Agricultural soils in the eastern parts of Denmark are generally categorized as clay containing, whereas the western parts are dominated by sandy soils. The same assessment evaluates copper to have a PEC/PNEC of 0.7-0.96. Though the risk of copper is evaluated lower in the present assessment, results are within the same range.

In conclusion zinc and copper may pose a high risk to the soil environment as a result of application of pig slurry to agricultural soils. The risk will be reduced when measures are implemented to reduce the content of zinc and copper in pig feed.
The copper and zinc concentrations resulting from sludge application is evaluated to pose low to medium risk to soil-living organisms.

#### *5.5.3.2 Arsenic (As)*

Arsenic concentrations are only available for sludge samples. Arsenic is present in sludge in the low mg/kg range. Estimated PEC in soil for arsenic after 100 years of sludge application is 0.14 mg/kg. A typical arsenic concentration in natural (uncontaminated) soil is 5 mg/kg and soil concentration is estimated to increase with approximately 3% over 100 years due to sludge application. Studies have shown that arsenic from sewage sludge can be taken up by agricultural plants (López-Rayo et al. 2016). Removal of arsenic via plant-uptake has not been taken into account, which may have lead to an overestimation of PEC.
Several studies have been conducted on the chronic effects of arsenic to soil organisms, including microorganisms, plants, invertebrates, mammals, birds, and hence the PNEC is considered to be of high quality. The PNEC used in this assessment of 0.5 mg/kg (see Appendix D, Table 3, PART II) is based on added arsenic, assuming organisms are adapted to the natural background of arsenic. Resulting PEC/PNEC value after 10 and 100 years of sewage sludge application is 0.03 and 0.27 respectively.
Arsenic is evaluated to pose low to medium risk to soil-dwelling organisms due to soil amendment with sewage sludge.

#### *5.5.3.3 Boron (B)*

Concentration of boron has been analysed in sludge samples. Concentrations are approximately 50 mg/kg resulting in PEC of 0.7 mg/kg after 100 years of sludge application. Boron does not exist in the

environment as a free metal, but is present as a salt and hence will be present mainly in the water-phase. Natural background concentrations in soil are expected to be 2-270 mg/kg (see Table 5.3). Boron from sludge application is hence not expected to contribute significantly to the total soil concentration. Further boron is an essential nutrient for plants (and humans), and is up-concentrated in higher plants, indicating, that removal by plant uptake will affect actual soil concentrations.
PNEC for boron is based on spices sensitivity distribution. It is however unclear whether soil background concentrations or only the added amount of boron were taken into account when determining the nominal boron concentrations.

The resulting PEC/PNEC for boron after 100 years of sludge application is 0.12. Taking into account the uncertainties for determination of PEC and the fact that boron will mainly be present in the aquatic environment, this might be overestimating the risk. It should however be noted, that boron may be toxic to aquatic species (PNEC of 2.9 mg/L), and hence leaching of boron to aquatic environments should be prevented.

Boron is evaluated to pose a medium risk to soil-living organism as a result of sewage sludge application.

#### *5.5.3.4 Chromium (Cr)*

Chromium is only analysed in sludge samples. Chromium is one of the most abundant metals in Danish sewage sludge with mean concentrations of 333 mg/kg. Estimated PEC of chromium after 100 years of sludge application is 8.2 mg/kg. The natural concentration of chromium in Danish soils is in the range of 1-100 mg/kg, with typical levels of 15 mg/kg (Table 5.3). The expected increase in soil concentrations after 100 years of sludge application is around 50 %, but will vary significantly depending on the natural background level.
Chromium may be present in soil as either chromium (VI) or chromium (III). Toxicity of chromium (VI) is expected to be up to 1000 times higher than that of chromium (III). The EU risk assessment (European Chemicals Bureau 2005) states that once released into soil it is likely that much of the chromium (VI) present will be reduced to chromium (III), and further, that only a minor fraction (0.1-1%) of the total chromium in soil is available to plants and soil fauna, and hence PNEC can be based on toxicity of chromium (III) alone. Toxicity of chromium is well investigated, and PNEC is considered of high quality. It should however be noted that the bioavailability, and hence toxicity, of chromium is highly dependent on pH and can increase under acidic conditions.
The PEC/PNEC resulting from the present assessment for chromium (III) after 10 and 100 years sludge application is 0.01 and 0.13 respectively (see Appendix E, Table 4, PART II).

Chromium is evaluated to pose a low to medium risk to the soil environment after application of sewage sludge. It should however be considered, that chromium may display higher toxicity when applied to acidic soil environments.

#### *5.5.3.5 Mercury (Hg)*

Concentrations of mercury are only available for sludge samples. Mercury is generally present in relatively low levels, in sludge in levels around 1 mg/kg (see Table 5.3). The soil quality criteria of mercury set by Miljø- og Fødevareministeriet (2017a) and listed in Table 2.1 gives a limit of 0.8 mg/kg dm. The mean concentration in sludge used for PEC calculations in the present report can hence be considered a worst-case scenario. In the natural soil environment, the range of mercury is 0.01-0.4 mg/kg. PEC of mercury after 100 years of sludge application is estimated to reach concentrations of 0.02 mg/kg, which is an approximate increase of 0.3 % per year (see Table 5.3).
Toxicity of mercury is high and PNEC is set to 0.2 mg/kg (see Appendix D, Table 3, PART II), resulting in a medium high PEC/PNEC value of 0.12 after 100 years of sludge application (Appendix E, Table 4, PART II). Mercury is adsorbed to the organic fraction in the soil and is considered immobile, but may be mobilized as complex with chloride or hydroxyl ions. The toxicity of the individual complexes has not been taken into account. Direct ingestion of adsorbed metal, has not been taken into account in the estimation of PNEC.

Mercury is evaluated of medium risk to the soil environment after 100 years of sludge application to agricultural soil.

#### *5.5.3.6 Remaining metals (Cd, Co, Mo, Se, Ag, TI, U, V, Sb, Al, Ba, Pb, Ni, and Sn)*

The remaining metals are either present in low concentrations in sludge and slurry (cadmium, cobalt, molybdenum, selenium, silver, thallium, uranium, vanadium) or have low toxicity towards terrestrial organisms (antimony, aluminium, barium, lead, nickel, tin).
Levels of aluminium and cadmium are determined in slurry samples. All, with the exception of aluminium, is determined in sludge.

When comparing levels of cadmium in the two fertilizer types, concentrations are markedly higher in sludge. However due to the differences in application rates (and differences in P content), the resulting PECs after 100 years are in the same range: 0.02-0.04 mg/kg (Appendix C, Table 2, PART I). Resulting PEC is highest for application of cattle slurry and lowest for application of pig slurry. The natural background concentration is in the range 0.003-0.9 mg/kg (see Table 5.3).

Cadmium concentrations in sludge are regulated and the limit set to 0.8 mg/kg dw (see Table 2.1). The mean concentration in sludge samples used for PEC calculations is 1.3 mg/kg (Appendix B, Table 1, PART I). This indicates, that the calculated PEC after sewage sludge application is reflecting a worst-case scenario. Cadmium is toxic to organisms and PNEC for cadmium is relatively low (1.15 mg/kg) (Appendix D, Table 2, PART I). However, even if pig slurry is applied and cadmium is allowed to accumulate in soil for a period of 100 years, total levels (background of 0.9 mg/kg plus applied levels of 0.04 mg/kg) would still be below the PNEC for adverse effects on soil organisms.

Due to the intermediate PEC/PNEC (between 0.01 and 0.1) barium, lead, cadmium, cobalt, molybdenum, nickel, silver and vanadium is evaluated of low risk to the soil environment after application of sewage sludge to agricultural soils. Likewise are aluminium, cadmium and nickel after application of slurry.
Antimony, selenium, thallium, tin and uranium all had PEC/PNECs below 0.01 and are evaluated to pose no risk to the soil environment as result of sewage sludge application.

### 5.5.4 Risk characterization of organic chemicals (except medicines)

98 organic chemicals are assessed for their potential risk towards soil organisms. Of these only six are monitored in slurry, making a direct comparison of the cumulative risk of animal fertilizer and sewage sludge arbitrary. The cumulative risk of organic chemicals is illustrated in Figure 5.4 and 5.5 for compound groups and single contaminants respectively. For illustrative purposes only compound groups or single chemicals with PEC/PNEC $\geq$ 0.1 are included in the figures, compound groups or single chemicals are included collectively as *remaining org. contaminants*. Individual and summed PEC/PNEC values for organic contaminants are summarized in Appendix E, Table 4, PART II.

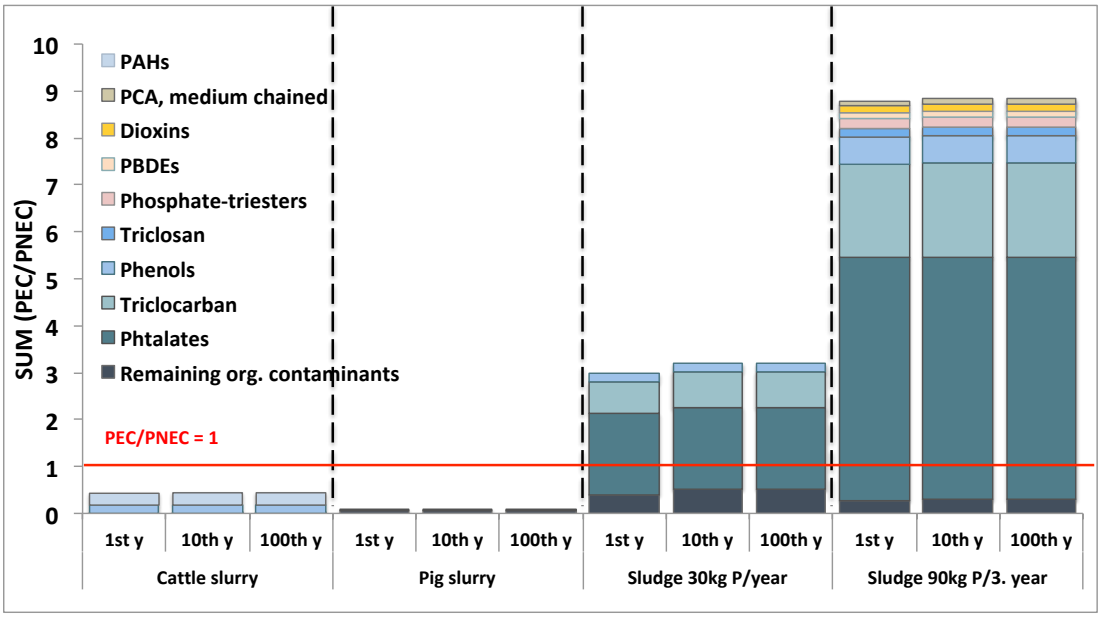

**Figure 5.4.** Calculated ∑PEC/PNEC values for groups of organic contaminants with PEC/PNEC ≥ 0.1. Compound groups with PEC/PNEC below 0.1 are summed as PEC/PNEC of remaining org. contaminants. Year 1, 10 and 100 refers to the 30 days initially after 1st., 10th and 100th year of application.

The ∑PEC/PNEC of organic chemicals was 0.43-0.44 for application of cattle slurry and 0.11-0.13 for application of pig slurry both initially after first, 10 and 100 years. The main compounds attributing to the toxicity are PAHs, NP2EO and nonylphenols for cattle slurry and solely PAH and NP2EO for pig slurry.

Based on the low ∑PEC/PNEC it is concluded that organic chemicals from slurry do not pose a risk to soil organisms. It should however be noted, that knowledge on organic chemicals in Danish slurry is sparse and hence, though expected to contain less residues from urban uses than sludge, slurry may contain substances not included in the present assessment.

For sludge, the ∑PEC/PNEC after 100 years of applications in amounts equal to 30 or 90 kg P/ha was approximately 3-4 and 9 respectively. For the latter scenario, the PEC/PNEC was ≥ 0.1 for 9 out of the 98 included organic compounds, these 9 compounds account for 93 % of the calculated risk. The compounds posing the highest risk in decreasing order are DOP > triclocarban > DHEA > NP2EO > tricresylphosphate > triclosan > 12378-PeCDD > NP1EO > PCAmedium. The only single compounds or compound groups with PEC/PNEC > 1 are phthalates (PEC/PNEC = 5.17, with DOP PEC/PNEC = 4.72) and triclocarban (PEC/PNEC = 1.99). Results further show, that ∑PEC/PNEC after 10 and 100 years are (close to) identical, indicating that the compounds contributing to the risk are not expected to accumulate in the soil environment.

The resulting ∑PEC/PNEC > 1 indicate that organic chemicals may reach soil concentrations after sludge application that may cause adverse effect to soil organisms. It should be taken into account, that the used PEC values refer to the point in time initially after application where soil concentrations are highest. Several of the organic compounds are of low persistence and hence will be degraded within days to months after application. Additionally, several of the PNEC values are derived by the application of an assessment factor of up to 1000 (with additional 10 for some very lipophilic compounds), which in some cases may be too conservative. This is discussed in further detail below. It should further still be kept in mind, that there might be organic contaminants present in matrix, not included in the present assessment.

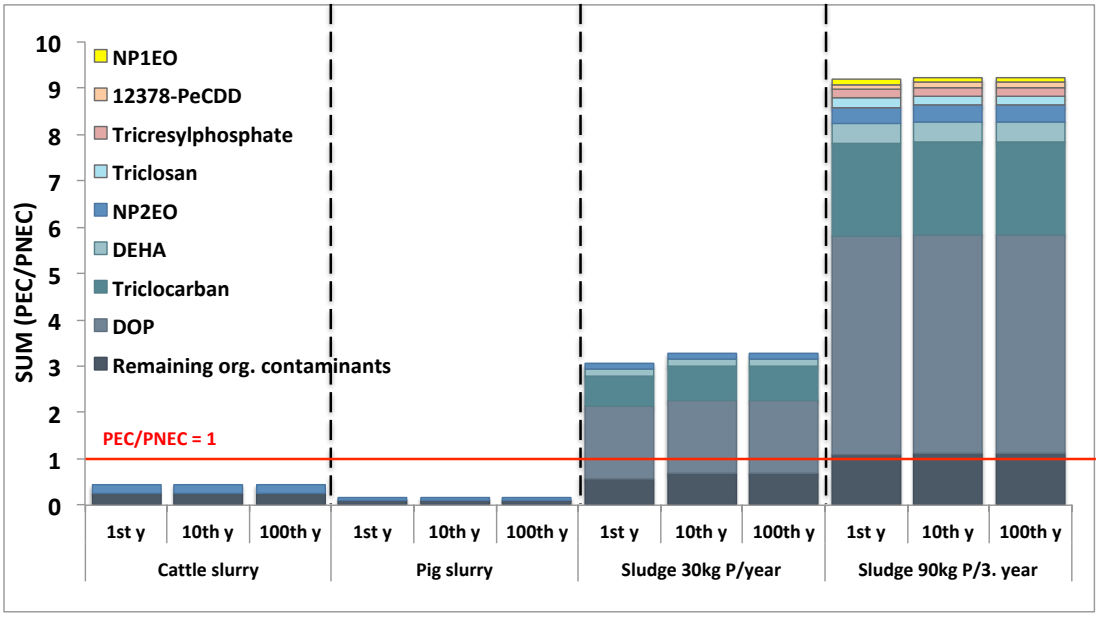


**Figure 5.5.** Calculated ∑PEC/PNEC values for single organic contaminants with PEC/PNEC ≥ 0.1. Compounds with PEC/PNEC below 0.1 are summed as PEC/PNEC of remaining org. contaminants. Year 1, 10 and 100 refers to the 30 days initially after 1st., 10th and 100th year of application.

### *5.5.4.1 Aromatic hydrocarbons*

Seven aromatic hydrocarbons are included in the present risk assessment. All seven are analyzed for in sludge samples, levels in slurry samples are not available. Aromatic hydrocarbons are produced during incomplete combustion of fossil fuels. They resemble PAHs structurally, but consist of one single aromatic ring.

∑PEC/PNEC of aromatic hydrocarbons is below 0.01 in all scenarios. Aromatic hydrocarbons are hence evaluated not to reach concentrations in soil after sludge application that may negatively affect soil organisms.

### *5.5.4.2 Chlorophenyls*

Three chlorophenyls are included in the present assessment, all of which have been analyzed for in sludge samples. None have been analyzed for in slurry. Pentachlorophenol has been used for conservation of wood, leather, and textiles. The main use of the remaining chlorophenyls is in production of pesticides.

Chlorophenyls are present in sludge in the µg/kg range and results show that the total risk of chlorophenyls is less than 0.001, even when assessing application of 90 kg P/3$^{rd}$ year.

Chlorophenyls are evaluated not to pose a risk to the soil environment after application of sewage sludge.

### *5.5.4.3 Dioxins and furans*

Seven dioxins and ten furans are included in the present assessment, due to their presence in sewage sludge. Information of dioxins and furans in slurry is not available.
Dioxins and furans are large groups of chemicals produced as by-products in industrial processes, e.g. during burning of organic material. Dioxins and furans are known for their high toxicity and measures have been taken to reduce the production of these compounds.

The risk evaluation resulted in a summed PEC/PNEC value of 0.13 and 0.22 for application of sludge as 30 kg P/year and 90 kg P/3$^{rd}$ year respectively. 12378-PeCDD accounts for approximately 50% of the calculated risk, due to its high toxicity (TEF of 1) and relatively high levels in sludge. As explained in section 5.4.2, the PNEC values for dioxins and furans are estimated based on their respective TEF relative to TCDD. The TEFs are established based on a human health perspective, and hence introduce uncertainties in the estimation of PNECs. Further TCDD toxicity is based on aquatic toxicity, which is available for three trophic levels, but not for terrestrial organisms. As dioxins and furans are highly lipophilic compounds (Kow > 5) an additional assessment factor of 10 is applied to the resulting PEC/PNEC, taking into account direct ingestion of soil-adsorbed compounds, which is otherwise not accounted for when estimating $PNEC_{soil}$ from $PNEC_{aq}$ (see also section 5.4.1).

In conclusion dioxins and furans are evaluated to pose a medium risk to soil organisms, as a result of soil amendment with sewage sludge. This evaluation is however highly uncertain due to the lack of toxicity information in the terrestrial environment, and it is recommended that more studies are performed in order to more accurately characterize the risk of these highly toxic compounds.

#### *5.5.4.4 Halogenated aliphatic and aromatic hydrocarbons (HAH)*

Seven halogenated aliphatic and aromatic hydrocarbons are included in the present risk assessment. These are chlorinated compounds used in industry as solvents or in production of pesticides. All seven HAHs are measured in sludge. Analyses of HAH are not available for slurry. Concentration mean is not available for all compounds, and hence max values are used for calculation of some PECs (see Appendix B, Table 1, PART II), which may result in an overestimation of the risk.
The summed risk of included HAHs is 0.006 and 0.02 for application of sludge as 30 kg P/year and 90 kg P/3$^{rd}$ year respectively. 2,5-dichloroaniline is the main HAH contributing to the risk and is accounting for 97 % of the total risk of HAHs in sewage sludge, possibly due to its high toxicity to aquatic organisms (see Appendix D, Table 3, PART II). Toxicity information of 2,5-dichloroaniline is not available for the terrestrial environment, and hence the $PNEC_{soil}$ value is estimated from $PNEC_{aq}$ introducing some uncertainties.

HAHs are evaluated to pose a low risk to soil living organisms due to application of sewage sludge.

#### *5.5.4.5 Linear alkylbenzene sulfonates (LAS)*

Linear alkylbenzene sulfonates are a class of anionic surfactants used as detergents in shampoo, toothpaste, laundry detergent etc. The concentrations of LAS compounds have been measured in both slurry and sludge, the individual compounds have however not been identified.

LAS are present in high levels in both slurry and sludge, and reach concentrations in the mg/kg range. The concentration in sludge is about 50 times higher than the levels in slurry. Estimated PECs in agricultural soil are 0.01, 0.01, 0.1 and 0.4 mg/kg initially after application of cattle and pig slurry, and application of sludge as 30 kg P/year and 90 kg P/3$^{rd}$ year respectively (see Appendix C, Table 2, PART II).

The $PNEC_{soil}$ for LAS is based on species sensitivity distribution and hence the $PNEC_{soil}$ is considered to be well founded.

The PEC/PNEC value for LAS is less than 0.005 for both slurry types. For application of sludge as 30 kg P/year and 90 kg P/3$^{rd}$ year the resulting PEC/PNEC is 0.03 and 0.09 respectively, indicating a low risk.

The quality criterion for LAS in sewage sludge used as fertilizer is set to 1300 mg/kg. The mean concentration of LAS used in the present assessment is 798 mg/kg, and hence approximately twice the amount could potentially be applied to agricultural soils. Using the maximum allowed concentration of LAS, results in a PEC/PNEC value of 0.18 for sludge applied as 90 kg P/3$^{rd}$ year.

A recent risk assessment by Jensen et al. (2001) evaluated the risk of LAS application to agricultural soils when applying an equivalent of 6 tonne sludge per hectare at maximum allowed LAS concentration (here set to 2600 mg/kg). At this higher application rate of sludge the resulting PEC/PNEC was calculated as 1.5. The authors further estimate, that the PEC/PNEC will drop to a level below 1 in 6-24 days post application, and hence conclude, that LAS does not pose a significant risk to terrestrial organisms or essential functions of agricultural soils as a result of normal sewage sludge amendment. Another risk assessment by the Norwegian Scientific Committee for Food Safety (Eriksen et al. 2009) also concluded, that LAS does not pose a risk to the soil environment due to sewage sludge application to agricultural soils, and this despite the fact that mean LAS concentrations in Norwegian sewage sludge is twice the concentration found in Danish sludge, and further exceeds the Danish quality criterion (Eriksen et al. 2009).

In agreement with previous risk assessment, it is concluded that LAS may pose only a low to medium risk to soil-living organisms after soil treatment with sewage sludge.

#### *5.5.4.6 Polyaromatic hydrocarbons (PAH)*

Concentrations of 22 polyaromatic hydrocarbons are measured in sludge samples. For perylene however no toxicity data is available, neither experimental or QSAR estimated, and hence the risk assessment for sludge is performed for the remaining 21 PAHs.

For slurry, the concentration of 19 PAH congeners, is available as the concentration of $\sum PAH_{19}$.

PAHs and aromatic hydrocarbons are formed during incomplete combustion of organic materials from e.g. motorized vehicles, chimneys and fires or during degradation of organic material in the environment.

PAH concentrations in sludge are approximately 5 and 10 times the concentration in cattle and pig slurry respectively. Resulting PEC is highest for sludge applied as 90 kg P/3$^{rd}$ year, followed by cattle slurry, sludge applied as 30 kg P/year and pig slurry (see Appendix C, Table 2, PART II).

A comparison and evaluation of the risk of PAHs from slurry and sludge, should be performed with care and take into account, that both PEC and PNEC for $\sum PAH_{19}$ in slurry has been estimated using average values of persistence and toxicity of the 21 known PAH congeners analyzed in sludge (see section 5.4.2). The $\sum$PEC/PNEC of PAHs in slurry is 0.3 and 0.06 for cattle and pig slurry respectively, whereas the $\sum$PEC/PNEC is calculated to 0.02 and 0.06 for application of sludge as 30 kg P/year and 90 kg P/3$^{rd}$ year respectively. Data on PAH toxicity on soil organisms were available for 9 out of 21 PAHs. Toxicity of the remaining PAH was estimated from toxicity towards aquatic species, either based on experimental data or on QSAR estimated toxicity, and further added an additional factor of 10 to account for direct ingestion of particle-bound compounds. $PNEC_{soil}$ were generally higher for PAH where experimental data for soil organisms were available, which might indicate a tendency towards overestimated toxicity when estimating $PNEC_{soil}$ from $PNEC_{aq}$.

PAHs from both types of animal slurry are evaluated to pose a low to medium risk to soil organisms. The concentration of PAHs in sludge was at the maximum allowed level of 3 mg/kg (see Table 2.1 and Appendix B, Tabel 1, PART II), and hence may be considered a worst-case scenario. In cases where sludge is applied there may be a medium risk of PAH induced toxicity to soil-living organisms. Based on the present assessment, the risk of PAH is evaluated to be highest when applying slurry from cattle.

#### *5.5.4.7 Polybrominated di-phenyl ethers (PBDEs)*

Twelve PBDEs are included for assessment, based on their presence and quantification in sewage sludge. Danish animal slurry has not been analyzed for PBDEs.

PBDEs are used as flame-retardants and added to products like textiles, plastics and foams.

Results from the risk assessment show that the summed PEC/PNEC for PBDEs is 0.08 and 0.14 for application of sludge as 30 kg P/year and 90 kg P/3$^{rd}$ year respectively (see Appendix E, Table 4, PART II). PBDE 99 is the congener contributing most to the risk, and is accounting for approximately 68 % of the summed risk of PBDEs. PBDE 99 is, together with PBDE 47, the most abundant in Danish sewage sludge.

$PNEC_{soil}$ for almost all of the included PBDEs is estimated from $PNEC_{aq}$. Terrestrial toxicity data is only available for PBDE 209, for which chronic toxicity data is available for three trophic levels. For the remaining PBDEs, $PNECs_{soil}$ are estimated from QSAR toxicity estimates for aquatic organisms and added an additional assessment factor accounting for direct ingestion of soil-bound chemicals. It is notable, that the $PNEC_{soil}$ for PBDE 209 is 100 to 1000 times higher than $PNECs_{soil}$ for the remaining PBDEs (see Appendix D, Table 2, PART II), which could indicate that calculating $PNEC_{soil}$ from $PNEC_{aq}$ for PBDEs might result in an over-conservative toxicity estimate.

PBDEs from application of sewage sludge are evaluated to pose a medium risk to the terrestrial environment. Due to the high uncertainties in estimating terrestrial toxicity, this conclusion should be used with great care.

#### *5.5.4.8 Polychlorinated biphenyls (PCB)*

Concentrations of polychlorinated biphenyls are determined in sludge samples, however only as Arochlor (formulated mixture of several PCB congeners) and polychlorinated terphenyl. It is likely that other PCBs are also present in sewage sludge. Data on PCB in Danish slurry is not available. PCBs have been used widely in building materials such as insulation and paint. Due to its toxicity and persistency, PCB is no longer used in Denmark, but can still be detected in the environment.

Results of the risk assessment show that at the present concentrations in sewage sludge, the included PCBs have a summed PEC/PNEC of less than 0.002, even when sludge is applied as 90 kg P/3$^{rd}$ year.

PCBs are evaluated not to pose a risk to the soil environment post application of sewage sludge to agricultural soils.

#### *5.5.4.9 Poly- and perfluoroalkylated substances (PFAS)*

Six congeners of the group poly- and perfluoroalkylated substances have been analyzed in sludge, no data is available on concentrations in slurry. PFAS are a large group of manufactured compounds, which have been widely used due to their water and grease repellent properties to make everyday products more resistant to stains, grease, and water. Especially perfluorooctane sulfonate (PFOS) has received

attention during the last decade, due to its suspected endocrine disruptive properties. It is currently registered in REACH Annex XVII and covered by the Stockholm convention.

Results of the risk assessment show a summed PEC/PNEC for PFASs of 0.003 and 0.005 for application of sludge as 30 kg P/year and 90 kg P/3$^{rd}$ year respectively. The Danish EPA has made a sum-criterion for PFAS congeners in soil, which is estimated based on the congener with the highest toxicity (PFOS). The criterion is based on human health information and is set to 0.4 mg/kg soil (Miljøministeriet 2015). The Norwegian Pollution Control Authority has proposed a guideline value for PFOS in soils of 0.1 mg/kg based upon effect studies on earthworms (Statens forurensningstilsyn 2006).
The calculated PEC of PFASs based on levels in Danish sewage sludge initially after application of sludge corresponding to 90kg P/3$^{rd}$ year is 0.03 µg/kg.
The $PNEC_{soil}$ values used in the present assessment are for the majority of PFASs estimated from aquatic toxicity data, either QSAR estimated or experimentally derived, and hence the PEC/PNEC is corrected with an additional factor accounting for direct ingestion of particle-bound substances. The derived $PNEC_{soil}$ for PFOA is very high (281 mg/kg) relative to $PNECs_{soil}$ calculated for remaining PFASs (in the range 0.03-0.4 mg/kg). PFOAs $PNEC_{soil}$ is calculated from an experimentally derived $PNEC_{aq}$, remaining $PNECs_{soil}$ is calculated from QSAR estimated aquatic toxicity. Whether toxicity to soil-living organism is underestimated for PFOA or overestimated for remaining PFASs, is unknown. This illustrates the high uncertainty in the risk estimation, and further illustrates the lack of toxicity information of PFAS towards soil-living organisms.

In conclusion, PFAS are evaluated to pose a low risk for soil-living organisms after application of sewage sludge to agricultural soils. It is however also acknowledged that soil toxicity of this contaminant group represents a knowledge gap.

#### *5.5.4.10 Phenols*

Six phenols are included in the present risk assessment. All six have been quantified in sludge, two in slurry (NP2EO and nonylphenol). Phenols are used in industry and in the processing of wood and plastics.
The concentration of NP2EO and nonylphenol is higher in sludge than slurry, but due to the higher P content and hence lower amount applied per hectare, the resulting PECs of the three fertilizer types are comparable.

Results from the risk assessment show that the summed PEC/PNEC is 0.1, 0.2, 0.2 and 0.6 for cattle and pig slurry, application of sludge as 30 kg P/year and 90 kg P/3$^{rd}$ year respectively (see Appendix E, Table 4, PART II). In sludge, the phenols contributing most to the summed risk are NP2EO, NP1EO,

phenol and nonylphenol listed in decreasing order, which mainly follows from the concentrations of the compounds in sludge. NP2EO accounts for approximately 60 % of the risk.

There are generally little information on toxicity of phenols to soil organisms, with the exception of bisphenol A, that are fairly well studied, however mainly for its endocrine disrupting effects. A terrestrial PNEC derived from studies on soil organisms was only available for half of the phenols, e.g. PNEC for phenol is based on a single EC50 value for *Eisenia foetida* with an assessment factor of 1000. The remaining PNECs were estimated based on aquatic toxicity and corrected with a factor 10 for ingestion of lipophilic compounds bound to soil materials (see Appendix D, Tabel 3, PART II).

In conclusion, it is acknowledged, that soil toxicity information on phenols is sparse, and renders the present assessment uncertain. However, based on the present risk assessment, phenols are evaluated to pose a medium risk to soil living organisms after application of slurry and sludge.

#### *5.5.4.11 Phosphate-triesters*

Four phosphate-triesters are included in the present assessment, of which all have been quantified in sludge and none in slurry. Phosphate-triesters are used as surface-active substances, plasticizers and flame-retardants.

The cumulative risk assessment of phosphate-triesters resulted in a summed PEC/PNEC of 0.07 and 0.20 for application of sludge as 30 kg P/year and 90 kg P/3$^{rd}$ year respectively. Tricresylphosphate accounts for more than 99 % of the summed risk. Mean concentrations of tricresylphosphate are not available, and hence a max concentration is used for the risk assessment. It is hence likely, that the average concentration of tricresylphosphate in sewage sludge is lower, and hence also the potential risk.

Toxicity data for phosphate-triesters is sparse. Terrestrial toxicity data is only available for tri-(2-chloroisopropyl)phosphate (TCPP), for the remaining three, $PNEC_{soil}$ is estimated from $PNEC_{aq}$.

Due to the low PEC/PNEC resulting from a (possibly) overestimated PEC, and despite the uncertainties in determining PNEC, phosphate-triesters from sewage sludge application is evaluated to pose a low risk to soil-living organisms.

#### *5.5.4.12 Phthalates*

Seven phthalates are included in the present assessment, based on their quantification in sludge samples. Data on the presence of two of these (DEHP and DBP) is also available for slurry samples. Phthalates are used in several plastic products to increase flexibility and longevity.

DEHP and DNP are the phthalates reaching the highest concentrations in sewage sludge. Of the two phthalates measured in slurry DEHP has the highest concentration, however only reaching one tenth of the level in sludge. Estimated PECs of DEHP initially after fertilizer application are comparable for slurry and sludge applied as 30 kg P/year (cattle 0.002, pig 0.001, low load sludge 0.003 mg/kg). Estimated PEC initially after sludge application as 90 kg P/3$^{rd}$ year is 0.01 mg/kg (see Appendix C, Table 2, PART II).

Results from the risk assessment show a summed PEC/PNEC for phthalates of 1.7 and 5.2 after application of sludge as 30 kg P/year and 90 kg P/3$^{rd}$ year application (see Appendix E, Table 4, PART II). PEC/PNEC for the two phthalates determined in slurry are comparable after slurry application (cattle = 0.001, pig = 0.0001) than PEC/PNEC after sludge application (30 kg P/year = 0.0003, 90 kg P/3$^{rd}$ year = 0.0008). It is unknown if phthalate congeners quantified only in sludge are also present in slurry.
Individual congeners with the highest PEC/PNEC (and with a PEC/PNEC > 0.01 for sludge applied as 90 kg P/3$^{rd}$ year) are DOP and DEHA, which in total account for more than 99 % of the phthalate associated risk. DOP alone reaches a PEC/PNEC of 4.72 and is one of the only compounds evaluated to have a risk on a substance-by-substance approach. DOP accounts for approximately 50 % of the summed risk of all included organic compounds.
The only phthalate, for which a sludge quality criterion has been set, is DEHP (see Table 2.1). The quality criterion is set to 50 mg/kg. The PECs used in the present assessment are estimated from a concentration in sludge of 16 mg/kg. Using the maximum allowed concentration, results in an estimated PEC/PNEC for DEHP of 0.002.

Very little information is available for terrestrial toxicity of phthalates. The PNECs used in the risk characterization are generally estimated from aquatic toxicity (see Appendix D, Table 3, PART II). The high PEC/PNEC for DOP might in part be due to the additional factor applied to DOP and DEHA to account for $PNEC_{soil}$ estimation from $PNEC_{aq}$ for compounds with Kow > 5. A comparative study of the impact of dimethyl phthalate (DMP, not included in the present assessment), DOP and DEP towards soil microorganisms showed lower toxicity of DOP compared with the less lipophilic phthalates (Kow of < 2.5) (Chen et al. 2013). In fact no significant impact was observed for DOP in concentrations up to 500 mg/kg. Information of DOP toxicity to invertebrates and other soil macro-organisms in soil is lacking from the literature. The lack of information is one of the reasons why these compounds have not been included (or fully included) in previous risk assessments of sewage sludge, which have mainly focused on DEHP (Jensen 2012; Eriksen et al. 2009; Smith 2009). Phthalates are expected to be fairly fast degraded in soils with reported half-lives for DOP of less than 50 days, and even as low as 5 days in aquatic systems under aerobic conditions (Agency for Toxic Substances and Disease Registry (ATSDR) 1997). Based on information of DOP gathered for the present assessment soil concentrations will reach levels below its PNEC after 27 days (see Figure 5.6).

DOP is listed under REACH Annex XVII, restricting manufacturing and use of the compound. It could be considered, if a quality criterion for sewage sludge should be developed for DOP.

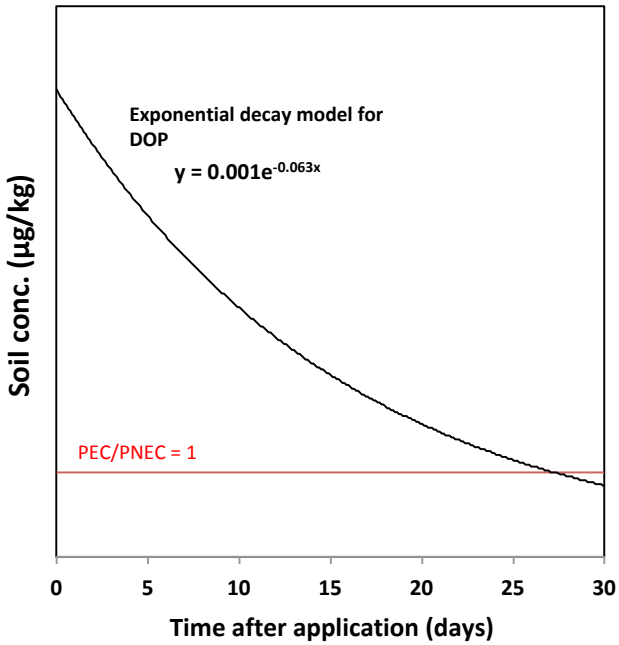


**Figure 5.6.** Disappearance of DOP in agricultural soils after application of sewage sludge, following a 1st order decay model. The red line indicates PEC/PNEC of 1.

In conclusion, based on the present information on phthalate toxicity towards mainly aquatic organisms, phthalates are evaluated to pose a risk to soil living organisms, especially DOP, in the month immediately after application of sewage sludge. It should however be taken into account, that toxicity information is hampered with uncertainties, and hence the present conclusion might be prove of a knowledge gap rather than actual risk.

Further, based on the available information on phthalates in slurry, these compounds are not expected to reach soil concentrations after slurry application, which may induce phthalate-associated toxicity.

#### *5.5.4.13 Polychlorinated naphtalenes (PCN)*

In the present assessment PEC of polychlorinated napthalenes in soil, is based on the summed level of PCNs in sludge, specific congeners have not been identified. PCNs are included in the present assessment due to the recommendation by the Danish ministry of Environment (Jensen 2012). Commercial PCNs are mixtures of up to 75 congeners and by-products. They have been used as insulation of electrical wires, but are no longer in use, and are mainly present in the environment as residues or due to the formation during thermal processes. The estimated PEC values are calculated from concentrations in sewage sludge from an British sewage treatment plant, due to lack of information on this group of contaminants in Danish sludge.

The PEC/PNEC for PCNs is below 0.001 for both application of sludge as 30 kg P/year and 90 kg P/3rd year. The $PNEC_{soil}$ used in the assessment are estimated from $PNEC_{aq}$ for Halowax (the most used mixture of PCN).

Assuming, that Danish sewage sludge contain PCNs in a range comparable to that in British sludge, PCNs from sewage sludge application to agricultural soils are not expected to pose a risk to soil-living organisms.

#### *5.5.4.14 Polychlorinated alkanes (PCA)*

For the present assessment polychlorinated alkanes are assessed as two groups of unspecified congeners based on their chain length (C10-13 and C14-17). The most common use for PCAs is as extreme-pressure, anti-wear additive in lubricants used for metal machinery e.g. cutting oil.
As for the PCNs, PCAs are included for risk assessment on recommendation by the Danish ministry of Environment (Jensen 2012). Due to the lack of data for Danish sewage treatment plants, PEC values are based on PCA concentrations in sewage sludge from the UK. It should be noted, that concentrations of PCAs in sediments are five times or higher in the UK compared to Denmark, which could indicate an overestimation of the risk when using these values (Larsen, Morten, and Sortkjær 2010; Nicholls, Allchin, and Law 2001).

Results of the cumulative risk assessment shows that PCAs have a summed PEC/PNEC of 0.04 and 0.12 for application of sludge as 30 kg P/year and 90 kg P/3rd year respectively. The short-chained PCAs are the most toxic, however almost 90 % of the calculated risk can be attributed to the medium-chained PCAs. This is due to the much higher abundance of medium-chained PCAs in sewage sludge compared with short-chained PCAs. The PNEC of medium-chained PCAs are based on chronic toxicity data for three trophic levels, and is hence considered to be of high quality.

Assuming, that PCA concentrations in Danish sewage sludge do not exceed that of British sludge, it is evaluated that, short- and medium-chained PCAs from sewage sludge application pose no and low risk to soil-living organisms respectively.

#### *5.5.4.15 Triclocarban and triclosan*

Triclocarban and triclosan concentrations have been quantified in sewage sludge. No data on levels in slurry is available.
Triclocarban and triclosan are biocides used for their antibacterial activity in several consumer products such as cosmetics, soap, toothpaste, cleaning products, textiles and paints. In a recent report on sewage sludge as fertilizer from The Danish EPA (Miljøministeriet 2012), it was recommended that triclocarban

and triclosan should be included in future monitoring and assessment programs due to their wide usage and high toxicity.
There is to our knowledge no information on actual levels of triclocarban in Danish sewage sludge, and the present PEC is estimated based on concentrations in sludge from an U.S. sewage treatment plant (of 51 mg/kg dw). Triclosan has however been analysed in sludge samples from both Denmark and the U.S., showing markedly higher concentrations in the US (DK 0.7-11 mg/kg dw, US 0.3-133 mg/kg dw, (Mogensen et al. 2008; U.S. EPA 2009)). It is possible that also triclocarban concentrations are higher in the US compared to Denmark, resulting in an overestimation of the risk.

There is little information on the toxicity of triclocarban and the majority of studies have focused on aquatic species. The PNEC used in this report is estimated from PNECaq and hence is hampered with uncertainties. The PNEC for triclosan on the other hand, is based on species sensitivity distribution, and is considered of high quality.

Results of the cumulative risk assessment show that triclocarban is one of the most risky compound in sewage sludge accounting for approximately 23 % of the total risk of organic compounds. With a PEC/PNEC of 0.75 and 1.99 in application of sludge as 30 kg P/year and 90 kg P/3$^{rd}$ year respectively, it is together with DOP, the only of the assessed organic compounds evaluated to reach environmental soil concentrations above its PNEC.

Assessment of triclosan resulted in a PEC/PNEC of 0.07 and 0.20 in application of sludge as 30 kg P/year and 90 kg P/3$^{rd}$ year respectively. Based on this, and taking into account that a PEC is calculated from the maximum concentration in sludge samples, and further, the high quality of PNEC value, triclosan is evaluated to pose a medium risk to soil organisms as a result of soil amendment with sewage sludge.

Based on the QSAR estimated properties of triclocarban, it is expected that triclocarban is degraded to a concentration below PNEC in the timespan between applications of sludge, even when sludge is applied yearly. Figure 5.7 shows the disappearance of triclocarban over time initially after application of sludge 90 kg P/3$^{rd}$ year. Calculated time to reach PEC/PNEC = 1 is 4.3 months (130 days), and hence the actual time where triclocarban might pose a potential risk is limited.

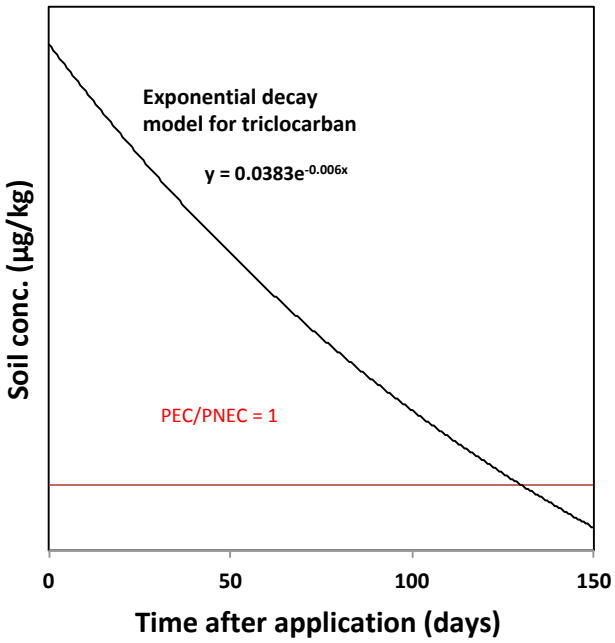


**Figure 5.7.** Disappearance of triclocarban in agricultural soils after application of sewage sludge, following a 1st order decay model. The red line indicates PEC/PNEC of 1.

This is in accordance with field studies, showing a decrease in soil concentrations of triclocarban from 44 ng/g to 22 ng/g over a period of 6 month (Gottschall et al. 2012). On the contrary another field study observed no discernable loss of triclocarban in agricultural soil over a three-year period after application of biosolids (Walters, McClellan, and Halden 2010). It should however be noted, that degradation of triclocarban is highly limited in aggregates of biosolids, probably due to decreased bioavailability (Gottschall et al. 2012). Decreased bioavailability may decrease risk due to limited exposure, but may also result in accumulation over time. In an agricultural soil treated with sewage sludge, measured soil concentrations of triclocarban were 0.027 mg/kg ww. Earthworms, sampled at this site, contained triclocarban in concentrations of 0.005 mg/kg ww, indicating a tendency towards bioaccumulation (Sherburne et al. 2016). Effects of the measured concentrations were not assessed.

Effects studies performed on agricultural soils receiving sewage sludge are sparse. In summary a study by Coors et al. (2016), evaluating abundance and diversity of soil organisms after soil amendment with sewage sludge, found no adverse effects on these endpoint during a 44-month period. The actual concentration of triclocarban was not reported in sludge or soil. In an extended long-term field study, referred to as CRUCIAL, the effects of sewage sludge on soil quality were evaluated. Sewage sludge was applied both at normal rates and at an accelerated rate. Results showed no adverse effect on the microbial community (Poulsen et al. 2013; Riber et al. 2014). Unfortunately concentrations of organic chemicals in sludge or soil were not determined.

In conclusion, application of sewage sludge might result in a PEC of triclocarban above PNEC for soil living organisms. This risk is however only present in the months immediately after application. The uncertainties in determination of both PEC and PNEC warrants additional information to improve the risk assessment for triclocarban in Danish soil environments. Based on the present assessment, triclocarban is evaluated to potentially causing harm to soil-living organisms as a result of application to agricultural soils, and hence it is recommended that concentration of triclocarban in Danish sludge be monitored.

### 5.5.5 Risk characterization of medicines and oestrogens

Medicines are developed with the intention of performing biological effects, and hence it is recognized, that medicines may also cause unwanted biological effects when introduced to the soil environment.
18 compounds belonging to the group of human or veterinary medicines were included in the present assessment and assessed for their potential risk towards soil organisms. Of these 2 and 5 were quantified in cattle and pig slurry respectively and 13 were quantified in sludge. There is no overlap in the quantified compounds for slurry and sludge, except for tetracycline being quantified in both pig slurry and in sludge, which might in part be explained by differences in human and veterinary medical practices.

Estrogens are endogenous steroid hormones produced in all mammals. Steroid hormones play a major role in growth, maturity, reproduction and several other vital functions.
Four estrogenic compounds are included in the present risk assessment covering the three natural estrogens (estrone E1, estradiol E2 and estriol E3) and the synthetic estrogen ethinylestradiol (EE2) used in e.g. contraceptives. Of these only estrone and estradiol are quantified in slurry, all are quantified in sludge. EE2 is not expected to be present in slurry.

There is a general lack of knowledge on the terrestrial toxicity of both medical compounds and estrogens. Of the 18 medical compounds included in the present risk assessment, only six $PNEC_{soil}$ were derived using toxicity information on soil living organisms. Eight $PNECs_{soil}$ were calculated from experimentally derived $PNEC_{aq}$ and the remaining four $PNECs_{soil}$ were calculated using QSAR estimated aquatic toxicity. In respect to estrogens, all $PNEC_{soil}$ were calculated from $PNEC_{aq}$.

The cumulative risk of medicines and estrogens is illustrated in Figure 5.8. For illustrative purposes, only chemicals with PEC/PNEC ≥ 0.1 are included in the figure.

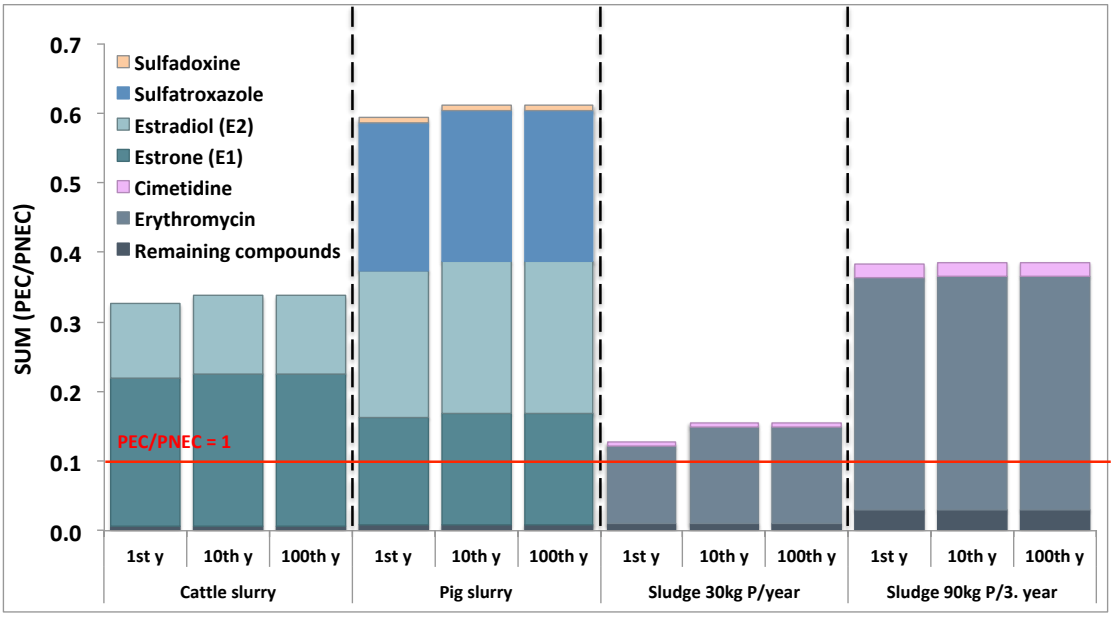


**Figure 5.8.** Calculated ∑PEC/PNEC values for medical residues and estrogens with PEC/PNEC ≥ 0.01. Compounds with PEC/PNEC below 0.01 are summed as PEC/PNEC of remaining compounds. Year 1, 10 and 100 refers to the 30 days initially after 1st., 10th and 100th year of application.

### *5.5.5.1 Medical compounds in slurry*

Only relatively few medical compounds have been analyzed for in Danish slurry, and the list of compounds included in the present assessment is hence limited. However, an extensive database by aus der Beek et al. (2016), allows for an overview of medical residues detected across selected European countries (here Germany, Denmark, Sweden and Norway). A search in the database revealed, that of the ten compounds reported in the highest amounts in slurry (in comparable units i.e. per weight), eight is either covered by the present assessment or excluded due to Danish slurry concentration being below limit of detection. The remaining two are sulfathiazole and abamectin.

Penicillins, sulfonamides and trimethoprim, and tetracyclines were the most sold therapeutic groups (per kg active ingredient) for Danish sows and piglets in 2015 (Statens Serum Institut 2017).

The included compounds quantified in slurry all belong to the group of sulfonamides, with the exception of tylosin and tetracycline. Sulfonamides are a group of antibacterial agents, often recommended for treatment of infections in domestic animals in Denmark. For increased effectiveness sulfonamides are sometimes given in combination with trimethoprim (Fødevarestyrelsen 2018).

The levels of medical residues are generally higher in pig slurry compared to cattle slurry. Several of the compounds included in the present assessment were blow limit of quantification in cattle slurry. In

Denmark domestic pigs account for 75% of the total antimicrobial consumption by animal species. For comparison cattle accounts for only 12%, which explains the difference in medical residues in their respective slurry (Statens Serum Institut 2017).
Sulfatroxazole is the compound with highest concentration in pig slurry. The presence and amount of antibiotic naturally depends on the degree of treatment in the specific facility, and sulfatroxazole was only above the limit of quantification in 5/17 samples (Schwærter and Grant 2003).

Is discussed above, toxicity information of medical compounds is sparse, and several $PNECs_{soil}$ were either calculated from experimental or QSAR estimated aquatic toxicity. This lack of information renders the present risk assessment uncertain.

The ∑PEC/PNEC of veterinary compounds was 0.01 for application of cattle slurry and 0.23 for application of pig slurry after 100 years. In pig slurry the sulfonamide sulfatroxazole accounts for more than 90 % of the cumulated risk.

The ∑PEC/PNEC < 1 indicate, that veterinary compounds are not expected to reach soil concentrations after application of slurry, that may cause adverse effects to soil organisms. It is however recognized that only a limited number of veterinary compounds are included for assessment, and further that, inclusion of additional compounds may potentially have great impacts on the results and the evaluation of the risk.

#### *5.5.5.2 Medical compounds in sludge*

Medical compounds in sludge are included on the basis of their quantification in sewage sludge in the screening program under NOVANA (Mogensen et al. 2008) or in the follow-up study (data taken from Jensen 2012). Several other pharmaceuticals were included in the screening, but were not detected and hence excluded from the present assessment. Taking these non-detectable compounds into account, the present assessment covers the top three most used pharmaceuticals in Denmark 2005, namely salicylic acid, paracetamol and ibuprofen (Mose Pedersen, Nielsen, and Halling-Sørensen 2007). Additional pharmaceuticals used in amounts above 20.000 kg are metformin and phenoxymethylpenicillin, for which the predicted fraction in sewage sludge is less than 5 % (QSAR estimation tool). Not only production volume, but also the amount of active compound excreted in human waste and subsequently adsorbed to sludge are of importance, and it is possible that medical compounds not included in this assessment is present in sludge and will reach the soil compartment as a result of sewage sludge application. Data from Sweden and Norway indicate that also the medical compounds ciprofloxacin, and norfloxacin may reach concentrations in sludge in the mg/kg range (aus der Beek et al. 2016). Ciprofloxacin was however included in a risk assessment on sewage sludge application by Eriksen (2009) concluding low risk when applying to agricultural soil (PEC/PNEC of 0.01-0.02). The risk

assessment by Eriksen (2009) included fourteen pharmaceutical compounds (out of 1414 on the Norwegian marked), based on the use, PEC, biological degradation and available toxicity information. Of the resulting fourteen, only tetracycline is also included in the present assessment. None of the selected compounds were evaluated as posing a risk to the soil environment (Eriksen et al. 2009).

For some medical compounds only the concentration range is available, and maximum concentrations were used as basis for PEC calculations (see Appendix B, Table 1, PART III), simulating a worst-case scenario. The medical compounds recorded with the highest concentration in Danish sludge are salicylic acid and paracetamol, in accordance with the high production of these compounds. It should however be noted, that these were among the compounds where maximum levels were used.

As also mentioned for veterinary compounds in slurry, toxicity information of medical compounds is sparse, and several of the used $PNECs_{soil}$ were either calculated from experimental or QSAR estimated aquatic toxicity, which renders the resulting risk estimation uncertain.

The present risk assessment results in ∑PEC/PNEC values for medical compounds of 0.16 and 0.39 for application of sludge as 30 kg P/year and 90 kg P/3$^{rd}$ year respectively. The main compounds contributing to the risk are erythromycin and cimetidine, accounting for more than 90 % of the cumulated risk. Erythromycin was quantified in 6 out of 20 samples in the range 15-69 ug/kg dw, and cimetidine in 10 out of 10 samples in the range 110-1200 ug/kg dw (Schwærter and Grant 2003). For both compounds PEC was estimated based on the maximum values, and hence can be considered a worst-case scenario. Cimetidine is further no longer in use in Denmark, and current levels are hence expected to be lower, than those used in the present assessment. As mentioned above, $PNEC_{soil}$ for both erythromycin and cimetidine is estimated from $PNEC_{aq}$ (see Appendix D, Table 3, PART III) and hence should be evaluated with care.

The risk evaluation of medical compounds in sludge is rendered uncertain due to the lack of knowledge of effects in the soil environment and it is recommended that more studies be performed in order to more accurately characterize the risk of this group of highly bioactive compounds to soil organisms. However, based on the available information, the assessed medical compounds and residues of these found in sewage sludge, is evaluated to pose a low risk to the soil environment as a result of sewage sludge application on agricultural fields. As for the veterinary compounds in slurry, it is however recognized that only a limited number of compounds are included for assessment, and that inclusion of additional compounds may potentially have great impact on results and evaluation of the risk.

#### *5.5.5.3 Estrogens*

Both males and females produce estrogens. Female production of estrogens varies depending on the reproductive status, and is highest in pregnant and lactating females. The three natural estrogens are produced in varying amounts and have different potencies towards their natural receptor. Estrogens are important regulators in the normal reproductive cycle, and hence unintended exposure to estrogens might cause endocrine disruptive effects.

To our knowledge concentrations of estrogens have not been quantified in Danish samples of either slurry or sludge. Concentrations are adopted from the US (slurry; estrone and estradiol) or Norway (sludge; estrone, estradiol, estriol and ethinylestradiol). Resulting PECs might differ from the actual concentrations of estrogens in Danish soils.
Concentrations of natural estrogens in slurry are up to 100-200 times higher than in sludge (see Appendix B, Table 1, PART IV), and calculated concentrations in soil after application are likewise minimum 100 timer higher after application of slurry than after application of sludge (see Appendix C, Table 2, PART III). This difference may however also be due to the difference in origin of data for slurry and sludge.

PEC values after application of pig slurry are based on values in slurry from non-farrowing sows. Especially levels of estradiol and estrone may be significantly higher in slurry from farrowing sows, more precisely 2 and 5 times higher respectively (Raman et al. 2004).
No data is available on the effects of estrogens on terrestrial organisms, and $PNEC_{soil}$ for estrogens are estimated from PNECaq and are hence uncertain.
The resulting ∑PEC/PNEC of estrogens is 0.33 and 0.38 after 100 years application of cattle and pig slurry respectively. For sewage sludge resulting ∑PEC/PNECs are below 0.01 for all scenarios.
Adopting slurry levels of estrogens from farrowing pigs results in PEC/PNEC of approximately 0.8, 0.4 and 1.24 for estrone, estradiol and ∑estrogens.

The risk evaluation of estrogens is highly uncertain due to the lack of information on presence of estrogens in fertilizer sources and the lack of toxicity information in the terrestrial environment. It is hence recommended, that more studies be performed in order to more accurately characterize the risk of estrogens. However, based on the current knowledge, estrogens from slurry and sewage sludge application are evaluated to pose low and medium risk to soil-living organisms respectively. In respect to pig slurry there may be a risk associated with use of slurry solely from farrowing pigs, where estrogen production is elevated.

# 6 ADDRESSING KNOWLEDGE GAPS

The current assessment highlights knowledge gaps that need to be addressed to further quantify the effects of recycling of different fertilizer sources. In general there is a lack of data on soil toxicity of emerging organic contaminants, as few chemical regulations require data on soil organisms. The current cumulative risk assessment therefore had to make very rough extrapolation from toxicity values based on aquatic organisms combined with rough estimations of bioavailable fractions of the toxicants, introducing a large degree of uncertainty in the risk assessment process. Developing databases on toxicity of organic contaminants in soil based on soil living and soil exposed organisms would therefore increase risk assessment certainty of individual substances considerably, making cumulative risk assessment and prioritization of soil pollutants more certain also. Ecotoxicological studies involving three or more levels of the soil food web would also allow a much greater confidence in determining the effects of persistent pollutants as for example di-n-octylphathalate (DOP) and triclocarban, which are responsible for more than half of the predicted toxicity of sewage sludge, but characterized by great uncertainty. A better determination of the ecotoxicological effects could thus allow use of a lower assessment factors.

These are 2 examples of ***'known unknowns'*** that we can manageably deal with, and thus increase the confidence in the ecotoxicological model predictions. There are other issues, such as lack of data on veterinary medicine in animal slurries and their transmission to crops, which is another knowledge gap that would require a large effort, and need integration in medicine approval protocols in order to remain effective in the future. Finally, there is the issue of possible decreasing concentrations of Cd (and Pb) in the soil and food system, due to decreased atmospheric combustion loading. The issue of Cd loading may be of high importance to clarify further, as there is a real concern that the current legislation is too lax, and should be tightened for reasons of public health. This in turn, could considerably impede recycling efforts.

However, as stated in the assessment, the a lack of knowledge of e.g. medicinal residues in animal slurries, and how they impact on soil and human health, may be an insurmountable barrier to realistically address in this day and age. Similarly, there may be unknown or uncharacterized compounds in the sewage sludges (e.g. microplastics), and while this assessment has attempted to take cocktail effects into account, it is at best a good approximation of the expected impact on soil organisms that has been given. This introduces the realm of the ***'unknown unknowns'***, which is commonly faced by decision makers when facing complex issues.

## 6.1 The use of integrated long-term experiments

We propose that one way of exploring and safeguarding for ***'unknown unknowns'***, is to invest in integrated long-term experiments. One example of an existing relevant and unique platform for research is the integrated long-term experiment 'CRUCIAL'[Ψ], developed at Copenhagen University, in which different types of waste and animal fertilizer has been applied in high or even excessive amounts to test if they pose a threat to the ecosystem integrity. The CRUCIAL long-term field experiment is located at an experimental farm of University of Copenhagen, situated 20 km west of Copenhagen, Denmark (55° 40´N, 12° 18´E) on a sandy loam (Magid et al., 2006). The field experiment was established in 2003 with 39 plots of 891 $m^2$ each. Each plot is separated from the neighbouring plots by 3 m wide strips of grass in order to avoid movement of soil between treatments. Treatments include urban fertilisers (human urine, sewage sludge, and composted municipal household waste), traditional manures (cattle slurry, cattle manure, deep litter, NPK) as well as unfertilised controls – one in which grain crops are undersown with white clover.

Application rates were adjusted to supply a modest input of N (equivalent to approximately 100 kg N $ha^{-1}$ $year^{-1}$ depending on the crop grown) using single sources and annual application rates were adjusted to take mineral N fertilizer equivalents (MFE) into account. This has caused substantial differences in the amounts of P applied, and thus differences in the 'equivalent number of years' of P supply (López-Rayo et al., 2016). Accelerated rates were also included in some treatments aiming at supplying three times the normal N level. In Danish agriculture use of sewage sludge and composted household waste is regulated on the basis of the P content, and the annual application is limited to 30 kg P $ha^{-1}$. In the CRUCIAL long-term field experiment we have intentionally breached this legal limitation, in order to rapidly move towards 'worst case' scenarios for waste recycling through acceleration. Thus, some of the fertilisation regimes applied have inevitably led to over-fertilisation with P and unbalanced inputs compared to good agricultural practices, resulting in some cases in a P supply equivalent to > 150-200 years (López-Rayo et al., 2016). A number of studies from CRUCIAL have documented that human urine, sewage sludge and composted household waste are beneficial and safe for soil fertility building.

López-Rayo et al. (2016) found that long-term amendment of urban and animal wastes equivalent to more than 100 years of application had minimal effect on plant uptake of potentially toxic elements. They investigated the effect of the different animal and urban waste treatments in CRUCIAL after ten years of experimentation, on oat yield and on concentrations of heavy metals in oat grain harvested in the field and pea plants grown in a pot trial using soil from the CRUCIAL field trial. They found all

[Ψ] CRUCIAL - Closing the Rural-Urban nutrient Cycle - Investigations through Agronomic Long-term experiments

animal and urban waste fertilizers to be suitable for fertilization purposes, although some of them would result in unbalanced nutrient inputs if used as single sources.

Using pyrosequencing, Poulsen et al. (2013) found no major changes in the bacterial community composition due to different fertilizer treatments, demonstrating a high robustness of the soil microbiota. However, some differences were observed e.g. Cyanobacteria were most frequent in the unfertilized soil, in comparison to the soils treated with nitrogen containing fertilizers and Firmicutes had higher occurrence in the soil with the composted household waste compared to all other treatments. Subsequently, Riber et al (2014) explored the immediate and long-term impact on bacterial communities in soil amended with animal and urban organic waste fertilizers using pyrosequencing and screening for horizontal transfer of antibiotic resistance. Bacterial community structure at phylum level remained mostly unaffected. Actinobacteria, Proteobacteria and Chloroflexi were the most prevalent phyla significantly responding to sampling time, but not fertilizer treatment. Seasonal changes seemed to prevail with decreasing bacterial richness in week 9 followed by a significant increase in week 29 (springtime). The Pseudomonas population richness seemed temporarily affected by fertilizer treatments, especially in sludge and compost-amended soils. Fertilizer amendment had a transient impact on the resistance profile of the soil community; abundance of resistant isolates decreased with time after fertilizer application (3 weeks), but persistent strains appeared multiresistant, also in unfertilized soil. Finally, the ability of a P. putida strain to take up resistance genes from indigenous soil bacteria by horizontal gene transfer was present only in week 0, indicating a temporary increase in prevalence of transferable antibiotic resistance genes. These studies of microbial diversity, have so far demonstrated a resilience of the soil microbiota and the soil ecosystem, and that the microbes introduced with the waste were either not viable or rapidly lost their antibiotic resistance traits.

Peltre et al. (2015) measured energy use for tillage with conventional spring tillage tines, as well as bulk density, soil texture and SOC content, and documented that repeated soil application of organic waste amendments reduced draught force and fuel consumption for soil tillage. Lekfeldt et al. (2017) assessed heavy metal leaching from intact soil columns. The leaching of Zn, Cd, and Co was not significantly increased in urban waste-fertilized treatments. Since leaching of Cr and Pb was strongly linked to the level of colloid leaching, leaching of these metals was reduced in the urban waste treatments. The leaching of Cu was significantly increased in the treatments receiving organic waste products compared with the unfertilized control but remained below the permissible level following Danish drinking water guidelines. The leaching of Cu was controlled primarily by the topsoil Cu content and by the leaching of dissolved organic carbon (DOC). Overall, the results presented did not raise concern regarding the agricultural use of urban waste products in agriculture as long as the relevant guidelines are followed. All things considered, so far negative effects, apart from an undesirable loss of nutrients through leaching, have not been seen. A number of studies have emerged from this facility and have been cited

in previous sections in this report, and there are also some ongoing preliminary studies worth mentioning.

### 6.2 A study on microplastics and earthworms in CRUCIAL

Plastics and microplastics are part and parcel of the urban waste stream, and appear in sorted solid organic waste as well as the sewage water. In a collaboration with Copenhagen University, Annemette Palmqvist and others (from Roskilde University) are examining microplastics and how they affect the behaviour and fertility of earthworms across treatments having received e.g. NPK, cattle manure, sewage sludge and composted household waste. The selected urban waste treatments had been given excessive amounts of these wastes, equivalent to 150-200 years of 'legal' application over a period of ca. 15 years. Recently an MSc thesis (Karling, 2018) has come out.

In this study Karling examined the earthworms found in the CRUCIAL trial, and the earthworms were divided according to functional groups and differences in distribution between the treatments. There was no significant effect of microplastics on the viability or reproduction of the earthworms (*Eisenia veneta*). This indicates that microplastics is not a threat to epigenetic earthworms. There were no significant effects of fertilizer types on earthworms in relation to body volume, burial time, hatching time or number of hatched cocoons. There were, however, significant differences in cocoon production. The treatment with accelerated composted household waste had the highest cocoon production while at the same time, by far the highest soil organic matter content. Thus, cocoon production was significantly higher compared to the sewage sludge treatment.

In addition, there were also significant differences in weight change. Here, the sewage sludge treatment had the highest positive weight gain (significant). The results of the weight change indicate a resource remodelling strategy where the earthworms either gain weight (highest for treatment sewage sludge, low for composted household waste) or produce cocoons (low number sewage sludge, high number for composted household waste). In both the reproduction experiment and field trial, there were results indicating different nutrient availability for the earthworms between the two alternative fertilizer types. It appears that sewage sludge has higher nutrient availability than composted household waste, which could explain the significantly higher number of earthworms in the field trial compared to what was found in the sewage sludge treatment. The soil organic matter content was not the determining factor in the field trial. The results indicate that the important factor for predicting impact on earthworm diversity and viability is the nutritional value of the resources, and not the content of microplastics.

### 6.3 A study on health of other soil living organisms in CRUCIAL

Vuaille (2017) attempted to determine the extent to which the application of organic waste had impacted biodiversity and soil quality in the CRUCIAL experiment. In this work, a reproductive capacity test in soil samples using nematode cultures (*Caenorhabditis elegans*) and enchytraeds (*Enchytraeus crypticus*) was applied to determine whether either worm cultures could be affected by the treatments applied to the plots.

Analysis of the results led to two main conclusions.

First, the abundance of nematodes and enchytraeids (extractions results and reproductive capacity tests) was not only governed by the organic matter content. Soil texture, moisture content and soil organic matter were also important factors, making sewage sludge plots and, to a lesser extent, cattle manure plots the most favourable for development of nematodes and enchytraeids. Secondly, cattle manure and sewage sludge appeared to have a different impact on the amount of nematodes extracted from soil samples. Organic matter was higher in cattle manure plots while nematode abundance was higher in the sludge plots.

### 6.4 A recommendation

To the best of our knowledge the CRUCIAL experimental site is unique, and no other place in the world has been developed to this extent, although other relevant points of reference can be found in Europe. We would recommend that this site is utilized to a greater extent in the coming years, in order to map the potential beneficial as well as detrimental effects the recycling of materials may have on diversity and health of soil living animals, and the impact on food quality originating from these long-term plots.

Finally, it needs to be recognized that there is only so much to be done on the basis of scientific studies. An important domain, which is outside the remit of this assessment, is the public acceptability and recognition of the need to recycle resources, that ‘in the best of all worlds’ would be free of unwanted substances, but in the real world is not.

# 7 Final conclusions

**Regarding risks to human health**

Based on the review, it is the expert opinion that sewage sludge does not represent a higher risk for propagation and transmission of antibiotic resistance than animal manure. It is not presently possible to quantify the human health risk associated with antibiotic resistance in soil, but we consider it most likely that other transmission pathways (e.g. human-human, animal-to-human or environmental transmission experienced by Danish residents while travelling) may be associated with a higher human health risk.

Among the risk factors discussed, PTEs are the best understood, and Cd and Pb are the most prominent of these in a Danish context, when it comes to direct effects on human health. It would seem highly relevant to further elucidate if the levels of Cd and Pb in crops are indeed on a declining path, due to the much lower combustion related atmospheric emissions over the last decade. We conclude that there is a low risk connected to PTE's in connection with human intake of crops fertilized with Danish sewage sludge. Finally, it is considered unlikely that veterinary medicinal residues in pig and cattle slurry are of concern for human health, and it is concluded that veterinary and human medicinal residues in sewage sludge are of low concern.

**Regarding risks to the soil environment**

The presence of compounds in animal slurry and sludge show very little overlap, thus making a direct comparison of the cumulative risk of animal fertilizer and sewage sludge somewhat arbitrary. The lack of overlap is probably due to the differences in origin, but also due to historical differences in the monitoring effort.
The cumulative risk assessment concluded, that there might be a potential risk of repeated use of animal slurry and sewage sludge in all fertilizer scenarios, present in the days initially after application, while pig slurry constitutes a higher chronic risk factor, due to the rather high levels of Cu and Zn.

Based on the low ∑PEC/PNEC it was concluded that organic chemicals, medical residues and estrogens from slurry pose a no or low risk to soil organisms. It should however be noted, that knowledge on organic chemicals in Danish slurry is sparse and hence, though expected to contain less residues from urban uses than sludge, slurry may contain substances not included in the present assessment.

Evaluation of sewage sludge use as fertilizer showed potential toxicity of phthalates and triclocarban. Conclusions are however uncertain due to the lack of both toxicity information, as well as specific Danish measurements of concentrations of some of these compounds. It is hence recommended that these compounds be monitored, at least until further knowledge may discard any uncertainties.

As a final note, it should be mentioned that, as toxicity values of the majority of the organic chemicals towards soil organisms are scarce, values from non-soil organisms or from computational estimations has been used together with large safety-factors (up to a factor 1000). The cumulative risk may therefore be inflated by these uncertainties and the calculations should be verified by experimental data. There are so far no indications from field monitoring studies where contemporary Danish sludge and manure have been used in parallel suggesting adverse effects on the soil biota compared to fields receiving mineral fertilizers.

Overall, we conclude that sewage sludge from contemporary Danish society does not constitute a higher risk than pig slurry.

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# APPENDICES

APPENDIX A: Abbreviations list

APPENDIX B: List of substance concentrations in slurry and sludge

APPENDIX C: Calculated predicted environmental concentrations (PECs)

APPENDIX D: Derived predicted no effect concentrations (PNECs)

APPENDIX E: Calculated PEC/PNEC values

# APPENDIX A

List of substance abbreviations used in Appendix tables, in order of appearance.

AHC = Aromatic hydrocarbons
HpCDD = Heptachlorodibenzo-p-dioxin
123478-HxCDD = 1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin
123678-HxCDD = 1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin
12378-PeCDD = 1,2,3,7,8-Pentachloridibenzo-p-dioxin
123789-HxCDD = 1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin
TCDD = 2,3,7,8-Tetrachlorodibenzo-p-dioxin
OCDD = Octachlorodibenzo-p-dioxin
1234678-HpCDF = 1,2,3,4,6,7,8-Heptachlorodibenzo-p-furan
123478-HxCDF =1,2,3,4,7,8-Hexachlorodibenzo-p-furan
1234789-HpCDF =1,2,3,4,7,8-Heptachlorodibenzo-p-furan
123678-HxCDF =1,2,3,6,7,8-Hexachlorodibenzo-p-furan
12378-PeCDF =1,2,3,7,8-Pentachlorodibenzo-p-furan
123789-HxCDF =1,2,3,7,8,9-Hexachlorodibenzo-p-furan
234678-HxCDF =1,2,3,4,7,8-Hexachlorodibenzo-p-furan
23478-PeCDF = 2,3,4,7,8-Pentachlorodibenzo-p-furan
2378-TCDF = 2,3,7,8-Tetrachlorodibenzo-p-furan
OCDF = Octachlorodibenzo-p-furan
HAH = Halogenated aromatic and aliphatic hydrocarbons
LAS = Linear alkylbenzene sulphonate
PAH = Polyaromatic hydrocarbons
PBDE = Polybrominated diphenyl ether
PCB = Polychlorinated biphenyls
PFSA = Poly- and perfluorinated alkylated substances
PFOS = Perfluorooctane sulfonate
PFDA = Perfluorodecanoic acid
PFNA = Perfluorononanoic acid
PFOSA = Perfluorooctanesulfonamide
PFOA = Perfluorooctanoic acid
PFUnA = Perfluoroundecanoic acid
NP2EO = Nonylphenol-diethoxylate
NP1EO = Nonylphenol-monoethoxylate
TCPP = Tri-(2-chloroisopropyl)phosphate
BBP = Benzylbutylphthalate
DOP = Di-n-octylphathalate
DEHA = Di(2-ethylhexyl)adipate
DEHP = Di(2-ethylhexyl)phthalate
DBP = Dibuthylphthalate
DEP = Diethylphthalate
DNP = Diisononylphthalate
PCN = Polychlorinated naphtalenes
PCA = Polychlorinated alkanes
NSAID = Non steroidal anti-inflammatory drug

# APPENDIX B

Levels of selected compounds in slurry and sludge. For specific references, the reader is referred to the main report.

**Table 1. PART I.** Mean (or *max*) concentrations of metals in slurry from cattle or pigs and in sewage sludge. All concentrations are given per dry weight.

| Compound | Cattle slurry | Pig slurry | Sewage sludge | |
|---|---|---|---|---|
| Antimony (Sb) | | | 3.5 | mg/kg |
| Aluminium (Al) | 664 | 330 | | mg/kg |
| Arsenic (As) | | | 7.5 | mg/kg |
| Barium (Ba) | | | 351 | mg/kg |
| Lead (Pb) | | | 45 | mg/kg |
| Boron (B) | | | 49 | mg/kg |
| Cadmium (Cd) | 0.370 | 0.370 | 1.3 | mg/kg |
| Copper (Cu) | 64.2 | 1298 | 28 | mg/kg |
| Cobalt (Co) | | | 5.4 | mg/kg |
| Chromium (Cr) | | | 333 | mg/kg |
| Mercury (Hg) | | | 1 | mg/kg |
| Molybdenum (Mo) | | | 7.2 | mg/kg |
| Nickel (Ni) | 6.3 | 10.2 | 26 | mg/kg |
| Selenium (Se) | | | 3.2 | mg/kg |
| Silver (Ag) | | | 4.1 | mg/kg |
| Thallium (Tl) | | | 170 | µg/kg |
| Tin (Sn) | | | 16 | mg/kg |
| Uranium (U) | | | 9.2 | mg/kg |
| Vanadium (V) | | | 18 | mg/kg |
| Zinc (Zn) | 231.8 | 3246 | 767 | mg/kg |

**Table 1. PART II**. Mean (or *max*) concentrations of organic contaminants in slurry from cattle and pig slurry and sewage sludge. All concentrations are given per dry weight.

| Compound group | Compound name | Cattle slurry | Pig slurry | Sewage sludge | |
|---|---|---|---|---|---|
| AHC | Benzene | | | *110* | µg/kg |
| AHC | Biphenyl | | | 127 | µg/kg |
| AHC | Ethylbenzene | | | 41 | µg/kg |
| AHC | Naphthalene | | | 121 | µg/kg |
| AHC | p-tert-butyltoluene | | | *21* | µg/kg |
| AHC | Toluene | | | 727 | µg/kg |
| AHC | Xylene | | | 207 | µg/kg |
| Chlorophenyls | 2,4-dichlorophenol | | | 101 | µg/kg |
| Chlorophenyls | 2,4,6-trichlorophenol | | | 11 | µg/kg |
| Chlorophenyls | 4-chloro-3-methylphenol | | | 15 | µg/kg |
| Dioxins | HpCDD | | | 98 | ng/kg |
| Dioxins | 123478-HxCDD | | | 1.8 | ng/kg |
| Dioxins | 123678-HxCDD | | | 6 | ng/kg |
| Dioxins | 12378-PeCDD | | | 1.9 | ng/kg |
| Dioxins | 123789-HxCDD | | | 3.2 | ng/kg |
| Dioxins | TCDD | | | 0.43 | ng/kg |
| Dioxins | OCDD | | | 753 | ng/kg |
| Furans | 1234678-HpCDF | | | 78 | ng/kg |
| Furans | 123478-HxCDF | | | 3.5 | ng/kg |
| Furans | 1234789-HpCDF | | | 3 | ng/kg |
| Furans | 123678-HxCDF | | | 3.1 | ng/kg |
| Furans | 12378-PeCDF | | | 2.1 | ng/kg |
| Furans | 123789-HxCDF | | | 1.7 | ng/kg |
| Furans | 234678-HxCDF | | | 3.5 | ng/kg |
| Furans | 23478-PeCDF | | | 3.5 | ng/kg |
| Furans | 2378-TCDF | | | 2.6 | ng/kg |
| Furans | OCDF | | | 315 | ng/kg |
| HAHs | Chloroform | | | *200* | µg/kg |
| HAHs | Dichloromethane | | | *230* | µg/kg |
| HAHs | Pentachloroethane | | | 0.32 | µg/kg |
| HAHs | Tetrachloroethylene | | | *8.5* | µg/kg |
| HAHs | Trichloroethylene | | | *200* | µg/kg |
| HAHs | 1,4-dichlorobenzene | | | 17 | µg/kg |
| HAHs | 2,5-dichloroanliline | | | 51 | µg/kg |
| LAS | Alkylbenzensulfonat | 15.5 | 15.6 | 789 | mg/kg |
| PAHs | 2-methylpyrene | | | 35 | µg/kg |
| PAHs | 1-methylpyrene | | | 28 | µg/kg |
| PAHs | 2-methylphenanthrene | | | 78 | µg/kg |
| PAHs | Acenaphthene | | | 47 | µg/kg |
| PAHs | Acenaphthylene | | | 38 | µg/kg |

**Table 1. PART II.** Continued

| | | | | | |
|---|---|---|---|---|---|
| PAHs | Antracene | | | 82 | µg/kg |
| PAHs | Benzo(a)anthracene | | | 134 | µg/kg |
| PAHs | Benzo(a)fluorene | | | 121 | µg/kg |
| PAHs | Benzo(a)pyrene | | | 144 | µg/kg |
| PAHs | Benzo(ghi)perylene | | | 156 | µg/kg |
| PAHs | Benzfluranthen b+j+k | | | 289 | µg/kg |
| PAHs | Benzo(e)pyren | | | 143 | µg/kg |
| PAHs | Chrysen/triphenyl | | | 210 | µg/kg |
| PAHs | Dibenz(ah)anthracen | | | 22 | µg/kg |
| PAHs | Dibenzothiophen | | | 20 | µg/kg |
| PAHs | Dimethylphenanthren | | | 44 | µg/kg |
| PAHs | Fluoranthen | | | 396 | µg/kg |
| PAHs | Fluoren | | | 82 | µg/kg |
| PAHs | Indeno(1,2,3-cd)pyren | | | 107 | µg/kg |
| PAHs | Perylene | | | 104 | µg/kg |
| PAHs | Phenanthrene | | | 391 | µg/kg |
| PAHs | Pyrene | | | 379 | µg/kg |
| PAH | PAH SUM(19) | 880 | 360 | | µg/kg |
| PBDEs | PBDE 17 | | | 3.5 | µg/kg |
| PBDEs | PBDE 28 | | | 2 | µg/kg |
| PBDEs | PBDE 47 | | | 29 | µg/kg |
| PBDEs | PBDE 49 | | | 3.3 | µg/kg |
| PBDEs | PBDE 66 | | | 1.3 | µg/kg |
| PBDEs | PBDE 85 | | | 2.6 | µg/kg |
| PBDEs | PBDE 99 | | | 32 | µg/kg |
| PBDEs | PBDE 100 | | | 6.3 | µg/kg |
| PBDEs | PBDE 153 | | | 3.5 | µg/kg |
| PBDEs | PBDE 154 | | | 3.3 | µg/kg |
| PBDEs | PBDE 183 | | | 3 | µg/kg |
| PBDEs | PBDE 209 | | | 363 | µg/kg |
| PCBs | Arochlor | | | 38 | µg/kg |
| PCBs | Polychlorineret terphenyl | | | 38 | µg/kg |
| PFSAs | PFOS | | | *11* | µg/kg |
| PFSAs | PFDA | | | 7.4 | µg/kg |
| PFSAs | PFNA | | | 1.5 | µg/kg |
| PFSAs | PFOSA | | | 15 | µg/kg |
| PFSAs | PFOA | | | 1.2 | µg/kg |
| PFSAs | PFUnA | | | *2.2* | µg/kg |
| Phenols | Bisphenol A | | | 990 | µg/kg |
| Phenols | NP2EO | 1.08 | 0.65 | 3.6 | mg/kg |
| Phenols | NP1EO | | | 1.89 | mg/kg |
| Phenols | Nonylphenoles | 1.6 | 1.7 | 8.5 | mg/kg |
| Phenols | Phenol | | | 19.8 | mg/kg |
| Phosphate-triesters | Tricresylphosphate | | | *930* | µg/kg |
| Phosphate-triesters | TCPP | | | 984 | µg/kg |
| Phosphate-triesters | Tributhylphosphate | | | 656 | µg/kg |

**Table 1. PART II.** Continued

| | | | | | |
|---|---|---|---|---|---|
| Phosphate-triesters | Triphenylphosphate | | | 65.8 | µg/kg |
| Phthalates | Benzylbutylphthalate (BBP) | | | 120 | µg/kg |
| Phthalates | Di-n-octylphathalate (DOP) | | | 1.6 | mg/kg |
| Phthalates | Di(2-ethylhexyl)adipate (DEHA) | | | *430* | µg/kg |
| Phthalates | Di(2-ethylhexyl)phthalate (DEHP) | 2.5 | 2.0 | 16.2 | mg/kg |
| Phthalates | Dibuthylphthalate (DBP) | 1300 | 0 | 340 | µg/kg |
| Phthalates | Diethylphthalate (DEP) | | | 69 | µg/kg |
| Phthalates | Diisononylphthalate (DNP) | | | 14.9 | mg/kg |
| Phenols | Octylphenol | | | 49 | µg/kg |
| PCN | Polychlorinated napthalenes (SUM35) | | | 0.083 | µg/kg |
| PCA, short chained | Polychlorinated alkanes C10-C13 | | | 42 | mg/kg |
| PCA, medium chained | Polychlorinated alkanes C14-C17 | | | 1800 | mg/kg |
| Biocide | Triclosan | | | *11* | mg/kg |
| Biocide | Triclocarban | | | 51 | mg/kg |

**Table 1. PART III.** Mean (or *max*) concentrations of medical compounds in slurry from cattle or pigs and in sewage sludge. All concentrations are given per dry weight.

| **Compound group** | **Compound name** | **Cattle slurry** | **Pig slurry** | **Sewage sludge** | |
|---|---|---|---|---|---|
| Antibiotic | Sulfadiazine | 0.0644 | 0.34 | <LOD | mg/kg |
| Antibiotic, | Sulfadimidine (Sulfamethazine) | 0.11 | <LOD | <LOD | mg/kg |
| Antibiotic | Sulfatroxazole | <LOD | 0.82 | | mg/kg |
| Antibiotic | Sulfadoxine | <LOD | 0.07 | <LOD | mg/kg |
| Antibiotic | Trimethoprim | <LOD | <LOD | *0.076* | mg/kg |
| Antibiotic | Tylosin | <LOD | 0.12 | <LOD | mg/kg |
| Antibiotic | Tetracycline | | 0.18 | 1.3 | mg/kg |
| Antibiotic | Amlodipine | | | *310* | µg/kg |
| Antibiotic | Cimetidine | | | *1200* | µg/kg |
| H2 receptor agonist | Erythromycin | | | *69* | µg/kg |
| Hypertension | Furosemid | | | *180* | µg/kg |
| Hypertension | Paracetamol | | | *2000* | µg/kg |
| Analgesic | Salicylic acid | | | *2800* | µg/kg |
| Analgesic | Sulfamethizole | <LOD | <LOD | 66.4 | µg/kg |
| Analgesic, NSAID | Ibuprofen | | | 10.27 | µg/kg |
| Analgesic, NSAID | Naproxen | | | 5.30 | µg/kg |
| Analgesic, NSAID | Ketoprofen | | | 5.9 | µg/kg |
| Antibiotic | Diclofenac | | | 10.7 | µg/kg |
| Antibiotic | Toltrazuril | | 0.114 | | mg/kg |

**Table 1. PART IV**. Mean (or *max*) concentrations of estrogens in slurry from cattle or pigs and in sewage sludge. All concentrations are given per dry weight.

| Compound group | Compound name | Cattle slurry | Pig slurry | Sewage sludge | |
|---|---|---|---|---|---|
| Estrogen | Estrone (E1) | 500 | 700 | 5.2 | µg/kg |
| Estrogen | Estradiol (E2) | 100 | 375 | 1.7 | µg/kg |
| Estrogen | Estriol (E3) | NA | NA | 1.07 | µg/kg |
| Estrogen | Ethinylestradiol (EE2) | NA | NA | 0.3 | µg/kg |

# APPENDIX C

**Table 2. PART I.** Kd values, respective references and estimated predicted environmental concentrations (PEC, mg/kg dw) of metals in soil after application of slurry or sludge. PECinit, PEC10, and PEC100 = PEC 30-day average initially after 1st, 10th and 100th year of application.

| | | | Cattle slurry | | | Pig slurry | | | Sludge 30kgP/ha/y | | | Sludge 90kg/ha/3y | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Compound name** | **Kd (L/kg)** | **Ref** | **PECinit** | **PEC10** | **PEC100** | **PECinit** | **PEC10** | **PEC100** | **PECinit** | **PEC10** | **PEC100** | **PECinit** | **PEC10** | **PEC100** |
| Antimony (Sb) | 401 | 1 | | | | | | | 8.75E-4 | 0.01 | 0.08 | 2.63E-3 | 0.01 | 0.08 |
| Aluminum (Al) | 29000 | 2 | 0.81 | 8.15 | 81.38 | 0.21 | 2.11 | 21.11 | | | | | | |
| Arsenic (As) | 83 | 3 | | | | | | | 1.88E-3 | 0.02 | 0.14 | 0.01 | 0.02 | 0.14 |
| Barium (Ba) | 960 | 2 | | | | | | | 0.09 | 0.88 | 8.52 | 0.26 | 0.79 | 8.44 |
| Lead (Pb) | 6511 | 1 | | | | | | | 0.01 | 0.11 | 1.12 | 0.03 | 0.10 | 1.11 |
| Boron (B) | 44 | 2 | | | | | | | 0.01 | 0.12 | 0.68 | 0.04 | 0.11 | 0.69 |
| Cadmium (Cd) | 3000 | 3 | 4.53E-4 | 4.53E-3 | 0.04 | 2.36E-4 | 2.36E-3 | 0.02 | 3.25E-4 | 3.25E-3 | 0.03 | 9.75E-4 | 2.92E-3 | 0.03 |
| Copper (Cu) | 1000 | 3 | 0.08 | 0.79 | 7.65 | 0.50 | 5.00 | 48.70 | 0.01 | 0.07 | 0.68 | 0.02 | 0.06 | 0.67 |
| Cobalt (Co) | 1265 | 1 | | | | | | | 1.35E-3 | 0.01 | 0.13 | 4.05E-3 | 0.01 | 0.13 |
| Chromium (Cr) | 4524 | 1 | | | | | | | 0.08 | 0.83 | 8.27 | 0.25 | 0.75 | 8.19 |
| Mercury (Hg) | 3000 | 2 | | | | | | | 2.50E-4 | 2.50E-3 | 0.02 | 7.50E-4 | 2.25E-3 | 0.02 |
| Molybdenum (Mo) | 40 | 1 | | | | | | | 1.80E-3 | 0.02 | 0.10 | 0.01 | 0.02 | 0.10 |
| Nickel (Ni) | 171 | 1 | 0.01 | 0.08 | 0.66 | 0.01 | 0.06 | 0.55 | 0.01 | 0.06 | 0.55 | 0.02 | 0.06 | 0.55 |
| Selenium (Se) | 449 | 1 | | | | | | | 8.00E-4 | 0.01 | 0.08 | 2.40E-3 | 0.01 | 0.07 |
| Silver (Ag) | 1900 | 2 | | | | | | | 1.03E-3 | 0.01 | 0.10 | 3.08E-3 | 0.01 | 0.10 |
| Thallium (Tl) | 9400 | 2 | | | | | | | 4.25E-5 | 4.25E-4 | 4.24E-3 | 1.28E-4 | 3.82E-4 | 4.19E-3 |
| Tin (Sn) | 3963 | 1 | | | | | | | 4.00E-3 | 0.04 | 0.40 | 0.01 | 0.04 | 0.39 |
| Uranium (U) | 422 | 1 | | | | | | | 2.30E-3 | 0.02 | 0.21 | 0.01 | 0.02 | 0.21 |
| Vanadium (V) | 7000 | 2 | | | | | | | 4.50E-3 | 0.04 | 0.45 | 0.01 | 0.04 | 0.44 |
| Zinc (Zn) | 422 | 3 | 0.28 | 2.82 | 26.54 | 2.16 | 21.45 | 201.68 | 0.19 | 1.91 | 17.92 | 0.58 | 1.72 | 17.78 |

**Table 2. PART II.** Registration number of respective QSAR reports (or alternative reference used), and resulting predicted environmental concentrations (PEC, mg/kg dw) of organic contaminants in soil after application of slurry or sludge. PECinit, PEC10, and PEC100 = PEC 30-day average initially after 1st, 10th and 100th year of application. When more than one QSAR report no is given, the average has been used in the calculation of PEC.

| | | | Cattle slurry | | | Pig slurry | | | Sludge 30kgP/ha/y | | | Sludge 90kg/ha/3y | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Compound group** | **Compound name** | **QSAR reg. no.** | **$PEC_{init}$** | **$PEC_{10}$** | **$PEC_{100}$** | **$PEC_{init}$** | **$PEC_{10}$** | **$PEC_{100}$** | **$PEC_{init}$** | **$PEC_{10}$** | **$PEC_{100}$** | **$PEC_{init}$** | **$PEC_{10}$** | **$PEC_{100}$** |
| AHC | Benzene | 71-43-2 | | | | | | | 3.58E-06 | 3.58E-06 | 3.58E-06 | 1.07E-05 | 1.07E-05 | 1.07E-05 |
| AHC | Biphenyl | 92-52-4 | | | | | | | 2.28E-05 | 2.28E-05 | 2.28E-05 | 6.84E-05 | 6.84E-05 | 6.84E-05 |
| AHC | Ethylbenzene | 100-41-4 | | | | | | | 4.15E-06 | 4.15E-06 | 4.15E-06 | 1.24E-05 | 1.24E-05 | 1.24E-05 |
| AHC | Naphthalene | 91-20-3 | | | | | | | 2.56E-05 | 2.60E-05 | 2.60E-05 | 7.68E-05 | 7.68E-05 | 7.68E-05 |
| AHC | p-tert-butyltoluene | 98-51-1 | | | | | | | 4.31E-06 | 4.34E-06 | 4.34E-06 | 1.29E-05 | 1.29E-05 | 1.29E-05 |
| AHC | Toluene | 108-88-3 | | | | | | | 4.94E-05 | 4.94E-05 | 4.94E-05 | 1.48E-04 | 1.48E-04 | 1.48E-04 |
| AHC | Xylene | 106-42-3 | | | | | | | 2.23E-05 | 2.23E-05 | 2.23E-05 | 6.69E-05 | 6.69E-05 | 6.69E-05 |
| Chloro-phenyls | 2,4-dichlorophenol | 120-83-2 | | | | | | | 2.20E-05 | 2.27E-05 | 2.27E-05 | 6.60E-05 | 6.60E-05 | 6.60E-05 |
| Chloro-phenyls | 2,4,6-trichlorophenol | 88-06-2 | | | | | | | 2.52E-06 | 2.86E-06 | 2.86E-06 | 7.57E-06 | 7.58E-06 | 7.58E-06 |
| Chloro-phenyls | 4-chloro-3-methylphenol | 59-50-7 | | | | | | | 3.27E-06 | 3.38E-06 | 3.38E-06 | 9.80E-06 | 9.80E-06 | 9.80E-06 |
| Dioxins | HpCDD | 35822-46-9 | | | | | | | 2.38E-08 | 4.71E-08 | 4.71E-08 | 7.14E-08 | 8.11E-08 | 8.13E-08 |
| Dioxins | 23478-HxCDD | EPISUITE | | | | | | | 4.37E-10 | 8.64E-10 | 8.65E-10 | 1.31E-09 | 1.49E-09 | 1.49E-09 |
| Dioxins | 123678-HxCDD | 57653-85-7 | | | | | | | 1.46E-09 | 2.88E-09 | 2.89E-09 | 4.37E-09 | 4.97E-09 | 4.98E-09 |
| Dioxins | 12378-PeCDD | 40321-76-4 | | | | | | | 4.62E-10 | 9.13E-10 | 9.13E-10 | 1.38E-09 | 1.57E-09 | 1.58E-09 |
| Dioxins | 123789-HxCDD | EPISUITE | | | | | | | 7.77E-10 | 1.54E-09 | 1.54E-09 | 2.33E-09 | 2.65E-09 | 2.65E-09 |
| Dioxins | TCDD | 1746-01-6 | | | | | | | 1.04E-10 | 2.06E-10 | 2.07E-10 | 3.13E-10 | 3.56E-10 | 3.56E-10 |
| Dioxins | OCDD | 3268-87-9 | | | | | | | 1.83E-07 | 3.62E-07 | 3.62E-07 | 5.49E-07 | 6.23E-07 | 6.25E-07 |
| Furans | 1234678-HpCDF | 38998-75-3 | | | | | | | 1.89E-08 | 3.74E-08 | 3.74E-08 | 5.68E-08 | 6.45E-08 | 6.46E-08 |
| Furans | 123478-HxCDF | 70648-26-9 | | | | | | | 8.50E-10 | 1.68E-09 | 1.68E-09 | 2.55E-09 | 2.89E-09 | 2.90E-09 |

**Table 2. PART II.** Continued

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Furans | 1234789-HpCDF | 55673-89-7 | | | | | | | 7.29E-10 | 1.44E-09 | 1.44E-09 | 2.19E-09 | 2.48E-09 | 2.48E-09 |
| Furans | 123678-HxCDF | 57117-44-9 | | | | | | | 7.53E-10 | 1.48E-09 | 1.49E-09 | 2.26E-09 | 2.56E-09 | 2.57E-09 |
| Furans | 12378-PeCDF | 57117-41-6 | | | | | | | 5.10E-10 | 1.00E-09 | 1.00E-09 | 1.53E-09 | 1.73E-09 | 1.74E-09 |
| Furans | 123789-HxCDF | 72918-21-9 | | | | | | | 4.13E-10 | 8.14E-10 | 8.14E-10 | 1.24E-09 | 1.40E-09 | 1.41E-09 |
| Furans | 234678-HxCDF | 60851-34-5 | | | | | | | 8.50E-10 | 1.68E-09 | 1.68E-09 | 2.55E-09 | 2.90E-09 | 2.90E-09 |
| Furans | 23478-PeCDF | 57117-31-4 | | | | | | | 8.50E-10 | 1.67E-09 | 1.67E-09 | 2.55E-09 | 2.89E-09 | 2.89E-09 |
| Furans | 2378-TCDF | 51207-31-9 | | | | | | | 6.31E-10 | 1.24E-09 | 1.24E-09 | 1.89E-09 | 2.14E-09 | 2.15E-09 |
| Furans | OCDF | 39001-02-0 | | | | | | | 7.65E-08 | 1.51E-07 | 1.51E-07 | 2.30E-07 | 2.61E-07 | 2.61E-07 |
| HAHs | Chloroform | EPISUITE | | | | | | | 1.96E-05 | 1.96E-05 | 1.96E-05 | 5.89E-05 | 5.89E-05 | 5.89E-05 |
| HAHs | Dichloro-methane | EPISUITE | | | | | | | 3.26E-06 | 3.26E-06 | 3.26E-06 | 9.79E-06 | 9.79E-06 | 9.79E-06 |
| HAHs | Pentachloro-ethane | 76-01-7 | | | | | | | 6.64E-08 | 6.70E-08 | 6.70E-08 | 1.99E-07 | 1.99E-07 | 1.99E-07 |
| HAHs | Tetrachloro-ethylene | 127-18-4 | | | | | | | 8.52E-07 | 8.52E-07 | 8.52E-07 | 2.56E-06 | 2.56E-06 | 2.56E-06 |
| HAHs | Trichloro-ethylene | 79-01-6 | | | | | | | 6.32E-06 | 6.32E-06 | 6.32E-06 | 1.89E-05 | 1.89E-05 | 1.89E-05 |
| HAHs | 1.4-dichloro-benzene | 106-46-7 | | | | | | | 3.29E-06 | 3.29E-06 | 3.29E-06 | 9.86E-06 | 9.86E-06 | 9.86E-06 |
| HAHs | 2.5-dichloro-anliline | 95-82-9 | | | | | | | 1.11E-05 | 1.14E-05 | 1.14E-05 | 3.32E-05 | 3.32E-05 | 3.32E-05 |
| LAS | LAS | (HERA, 2013; Jensen et al., 2001) | 1.29E-02 | 1.29E-02 | 1.29E-02 | 6.76E-03 | 6.76E-03 | 6.76E-03 | 1.34E-01 | 1.34E-01 | 1.34E-01 | 4.02E-01 | 4.02E-01 | 4.02E-01 |
| PAHs | 2-methylpyrene | 3442-78-2 | | | | | | | 8.03E-06 | 9.14E-06 | 9.14E-06 | 2.41E-05 | 2.41E-05 | 2.41E-05 |
| PAHs | 1-methylpyrene | 2381-21-7 | | | | | | | 6.43E-06 | 7.31E-06 | 7.31E-06 | 1.93E-05 | 1.93E-05 | 1.93E-05 |
| PAHs | 2-methyl-phenanthrene | 2531-84-2 | | | | | | | 1.70E-05 | 1.76E-05 | 1.76E-05 | 5.11E-05 | 5.11E-05 | 5.11E-05 |
| PAHs | Acenaphthene | 83-32-9 | | | | | | | 1.02E-05 | 1.05E-05 | 1.05E-05 | 3.07E-05 | 3.07E-05 | 3.07E-05 |
| PAHs | Acenaphthylene | EPISUITE | | | | | | | 6.84E-06 | 6.84E-06 | 6.84E-06 | 2.05E-05 | 2.05E-05 | 2.05E-05 |
| PAHs | Antracene | 120-12-7 | | | | | | | 1.88E-05 | 2.14E-05 | 2.14E-05 | 5.64E-05 | 5.65E-05 | 5.65E-05 |
| PAHs | Benzo(a)-Anthracene | 56-55-3 | | | | | | | 3.08E-05 | 3.50E-05 | 3.50E-05 | 9.23E-05 | 9.24E-05 | 9.24E-05 |

**Table 2. PART II**. Continued

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PAHs | Benzo(a)-fluorene | 238-84-6 | | | | | | | 2.64E-05 | 2.74E-05 | 2.74E-05 | 7.93E-05 | 7.93E-05 | 7.93E-05 |
| PAHs | Benzo(a)pyrene | 50-32-8 | | | | | | | 3.31E-05 | 3.76E-05 | 3.76E-05 | 9.92E-05 | 9.93E-05 | 9.93E-05 |
| PAHs | Benzo(ghi)-perylene | 191-24-2 | | | | | | | 3.58E-05 | 4.08E-05 | 4.08E-05 | 1.07E-04 | 1.08E-04 | 1.08E-04 |
| PAHs | Benzfluranthen b+j+k | 205-82-3, 205-99-2, 207-08-9 | | | | | | | 6.63E-05 | 7.55E-05 | 7.55E-05 | 1.99E-04 | 1.99E-04 | 1.99E-04 |
| PAHs | Benzo(e)pyren | 192-97-2 | | | | | | | 3.28E-05 | 3.74E-05 | 3.74E-05 | 9.85E-05 | 9.86E-05 | 9.86E-05 |
| PAHs | Chrysen/-triphenyl | 218-01-9 | | | | | | | 4.82E-05 | 5.49E-05 | 5.49E-05 | 1.45E-04 | 1.45E-04 | 1.45E-04 |
| PAHs | Dibenz(ah)-anthracen | 53-70-3 | | | | | | | 5.05E-06 | 5.75E-06 | 5.75E-06 | 1.51E-05 | 1.52E-05 | 1.52E-05 |
| PAHs | Dibenzo-thiophen | 132-65-0 | | | | | | | 3.60E-06 | 3.61E-06 | 3.61E-06 | 1.08E-05 | 1.08E-05 | 1.08E-05 |
| PAHs | Dimethyl-phenanthren | 16664-45-2 | | | | | | | 9.61E-06 | 9.95E-06 | 9.95E-06 | 2.88E-05 | 2.88E-05 | 2.88E-05 |
| PAHs | Fluoranthen | 206-44-0 | | | | | | | 9.09E-05 | 1.03E-04 | 1.03E-04 | 2.73E-04 | 2.73E-04 | 2.73E-04 |
| PAHs | Fluoren | 86-73-7 | | | | | | | 1.48E-05 | 1.48E-05 | 1.48E-05 | 4.43E-05 | 4.43E-05 | 4.43E-05 |
| PAHs | Indeno-(1,2,3-cd)pyren | 193-39-5 | | | | | | | 2.46E-05 | 2.80E-05 | 2.80E-05 | 7.37E-05 | 7.38E-05 | 7.38E-05 |
| PAHs | Perylene | EPISUITE | | | | | | | 2.39E-05 | 2.72E-05 | 2.72E-05 | 7.16E-05 | 7.17E-05 | 7.17E-05 |
| PAHs | Phenanthrene | 85-01-8 | | | | | | | 8.97E-05 | 1.02E-04 | 1.02E-04 | 2.69E-04 | 2.70E-04 | 2.70E-04 |
| PAHs | Pyrene | 129-00-0 | | | | | | | 8.70E-05 | 9.90E-05 | 9.90E-05 | 2.61E-04 | 2.61E-04 | 2.61E-04 |
| PAHs | PAH SUM(19) | Estimated from SUM PAH | 9.48E-04 | 9.88E-04 | 9.88E-04 | 2.02E-04 | 2.11E-04 | 2.11E-04 | | | | | | |
| PBDEs | PBDE 17 | EPISUITE | | | | | | | 8.03E-07 | 9.14E-07 | 9.14E-07 | 2.41E-06 | 2.41E-06 | 2.41E-06 |
| PBDEs | PBDE 28 | EPISUITE | | | | | | | 4.37E-07 | 4.52E-07 | 4.52E-07 | 1.31E-06 | 1.31E-06 | 1.31E-06 |
| PBDEs | PBDE 47 | 5436-43-1 | | | | | | | 7.04E-06 | 1.39E-05 | 1.39E-05 | 2.11E-05 | 2.40E-05 | 2.40E-05 |
| PBDEs | PBDE 49 | Estimated from PBDE 47 | | | | | | | 8.02E-07 | 1.59E-06 | 1.59E-06 | 2.40E-06 | 2.73E-06 | 2.74E-06 |
| PBDEs | PBDE 66 | Estimated from PBDE 47 | | | | | | | 3.16E-07 | 6.25E-07 | 6.25E-07 | 9.47E-07 | 1.08E-06 | 1.08E-06 |
| PBDEs | PBDE 85 | EPISUITE | | | | | | | 6.32E-07 | 1.25E-06 | 1.25E-06 | 1.89E-06 | 2.15E-06 | 2.16E-06 |
| PBDEs | PBDE 99 | 32534-81-9 | | | | | | | 7.77E-06 | 1.54E-05 | 1.54E-05 | 2.33E-05 | 2.65E-05 | 2.65E-05 |
| PBDEs | PBDE 100 | EPISUITE | | | | | | | 1.53E-06 | 3.03E-06 | 3.03E-06 | 4.59E-06 | 5.22E-06 | 5.23E-06 |

**Table 2. PART II**. Continued

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PBDEs | PBDE 153 | 68631-49-2 | | | | | | | 8.50E-07 | 1.68E-06 | 1.68E-06 | 2.55E-06 | 2.90E-06 | 2.90E-06 |
| PBDEs | PBDE 154 | PBDE 153 | | | | | | | 8.02E-07 | 1.59E-06 | 1.59E-06 | 2.40E-06 | 2.73E-06 | 2.74E-06 |
| PBDEs | PBDE 183 | PBDE 153 | | | | | | | 7.29E-07 | 1.44E-06 | 1.44E-06 | 2.19E-06 | 2.48E-06 | 2.49E-06 |
| PBDEs | PBDE 209 | 1163-19-5 | | | | | | | 8.82E-05 | 1.75E-04 | 1.75E-04 | 2.65E-04 | 3.01E-04 | 3.01E-04 |
| PCBs | Arochlor | 37680-65-2; 38444-90-5 | | | | | | | 8.70E-06 | 9.80E-06 | 9.80E-06 | 2.61E-05 | 2.61E-05 | 2.61E-05 |
| PCBs | Polychlorinated terphenyl | 84-15-1 | | | | | | | 8.30E-06 | 8.59E-06 | 8.59E-06 | 2.49E-05 | 2.49E-05 | 2.49E-05 |
| PFSAs | PFOS | 1763-23-1 | | | | | | | 2.67E-06 | 5.25E-06 | 5.26E-06 | 8.01E-06 | 9.08E-06 | 9.10E-06 |
| PFSAs | PFDA | 335-76-2 | | | | | | | 1.80E-06 | 3.56E-06 | 3.56E-06 | 5.39E-06 | 6.13E-06 | 6.14E-06 |
| PFSAs | PFNA | 375-95-1 | | | | | | | 3.64E-07 | 7.20E-07 | 7.21E-07 | 1.09E-06 | 1.24E-06 | 1.24E-06 |
| PFSAs | PFOSA | 754-91-6 | | | | | | | 3.64E-06 | 7.21E-06 | 7.21E-06 | 1.09E-05 | 1.24E-05 | 1.24E-05 |
| PFSAs | PFOA | 335-67-1 | | | | | | | 2.91E-07 | 5.75E-07 | 5.75E-07 | 8.74E-07 | 9.92E-07 | 9.94E-07 |
| PFSAs | PFUnA | 2058-94-8 | | | | | | | 5.34E-07 | 1.06E-06 | 1.06E-06 | 1.60E-06 | 1.82E-06 | 1.82E-06 |
| Phenols | Bisphenol-A | 80-05-7 | | | | | | | 2.16E-04 | 2.23E-04 | 2.23E-04 | 6.48E-04 | 6.48E-04 | 6.48E-04 |
| Phenols | NP2EO | 9016-45-9 | 9.54E-04 | 9.55E-04 | 9.55E-04 | 2.99E-04 | 2.99E-04 | 2.99E-04 | 6.49E-04 | 6.49E-04 | 6.49E-04 | 1.95E-03 | 1.95E-03 | 1.95E-03 |
| Phenols | NP1EO | 27986-36-3 | | | | | | | 3.41E-04 | 3.41E-04 | 3.41E-04 | 1.02E-03 | 1.02E-03 | 1.02E-03 |
| Phenols | Nonylphenole | 104-40-5 | 1.41E-03 | 1.41E-03 | 1.41E-03 | 7.83E-04 | 7.83E-04 | 7.83E-04 | 1.54E-03 | 1.54E-03 | 1.54E-03 | 4.61E-03 | 4.61E-03 | 4.61E-03 |
| Phenols | Phenol | 100-67-4 | | | | | | | 3.52E-03 | 3.52E-03 | 3.52E-03 | 1.06E-02 | 1.06E-02 | 1.06E-02 |
| Phosphate -triester | Tricresyl-phosphate | 78-30-8 | | | | | | | 2.03E-04 | 2.10E-04 | 2.10E-04 | 6.09E-04 | 6.09E-04 | 6.09E-04 |
| Phosphate -triester | TCPP | 13674-84-5 | | | | | | | 2.25E-04 | 2.52E-04 | 2.52E-04 | 6.75E-04 | 6.75E-04 | 6.75E-04 |
| Phosphate -triester | Tributhyl-phosphate | 126-73-8 | | | | | | | 9.55E-05 | 9.55E-05 | 9.55E-05 | 2.86E-04 | 2.86E-04 | 2.86E-04 |
| Phosphate -triester | Triphenyl-phosphate | 115-86-6 | | | | | | | 1.44E-05 | 1.49E-05 | 1.49E-05 | 4.31E-05 | 4.31E-05 | 4.31E-05 |
| Phthalate | BBP | 85-68-7 | | | | | | | 2.16E-05 | 2.16E-05 | 2.16E-05 | 6.49E-05 | 6.49E-05 | 6.49E-05 |
| Phthalate | DOP | 117-84-0 | | | | | | | 2.89E-04 | 2.89E-04 | 2.89E-04 | 8.66E-04 | 8.66E-04 | 8.66E-04 |
| Phthalate | DHEA | 103-23-1 | | | | | | | 6.26E-05 | 6.26E-05 | 6.26E-05 | 1.88E-04 | 1.88E-04 | 1.88E-04 |

**Table 2. PART II**. Continued

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Phthalate | DEHP | 117-81-7 | 2.21E-03 | 2.21E-03 | 2.21E-03 | 9.21E-04 | 9.22E-04 | 9.22E-04 | 2.92E-03 | 2.92E-03 | 2.92E-03 | 8.76E-03 | 8.76E-03 | 8.76E-03 |
| Phthalate | DBP | 84-74-2 | 9.27E-04 | 9.27E-04 | 9.27E-04 | | | | 4.95E-05 | 4.95E-05 | 4.95E-05 | 1.48E-04 | 1.48E-04 | 1.48E-04 |
| Phthalate | DEP | 84-66-2 | | | | | | | 1.23E-05 | 1.23E-05 | 1.23E-05 | 3.70E-05 | 3.70E-05 | 3.70E-05 |
| Phthalate | DNP | 70857-56-6 | | | | | | | 2.68E-03 | 2.68E-03 | 2.68E-03 | 8.03E-03 | 8.03E-03 | 8.03E-03 |
| Phenols | Octylphenol | 949-13-3 | | | | | | | 8.84E-06 | 8.84E-06 | 8.84E-06 | 2.65E-05 | 2.65E-05 | 2.65E-05 |
| PCN | PCN (SUM35) | 34588-40-4 | | | | | | | 1.90E-08 | 2.16E-08 | 2.16E-08 | 5.71E-08 | 5.72E-08 | 5.72E-08 |
| PCA, short chained | PCA C10-C13 | 63981-28-2 | | | | | | | 9.64E-03 | 1.10E-02 | 1.10E-02 | 2.89E-02 | 2.90E-02 | 2.90E-02 |
| PCA, medium chained | PCA C14-C17 | EPISUITE | | | | | | | 4.13E-01 | 4.70E-01 | 4.70E-01 | 1.24 | 1.24 | 1.24 |
| Biocide | Triclosan | EPISUITE | | | | | | | 2.52E-03 | 2.87E-03 | 2.87E-03 | 7.57E-03 | 7.59E-03 | 7.59E-03 |
| Biocide | Triclocarban | 101-20-2 | | | | | | | 1.17E-02 | 1.33E-02 | 1.33E-02 | 3.51E-02 | 3.52E-02 | 3.52E-02 |

**Table 2. PART III**. Registration number of respective QSAR reports (or alternative reference used), and resulting predicted environmental concentrations (PEC, mg/kg dw) of medical compound or estrogen in soil after application of slurry or sludge. PECinit, PEC10, and PEC100 = PEC 30-day average initially after 1st, 10th and 100th year of application.

| | | | Cattle slurry | | | Pig slurry | | | Sludge 30kgP/ha/y | | | Sludge 90kg/ha/3y | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Compound group** | **Compound name** | **QSAR reg. no.** | **PECinit** | **PEC10** | **PEC100** | **PECinit** | **PEC10** | **PEC100** | **PECinit** | **PEC10** | **PEC100** | **PECinit** | **PEC10** | **PEC100** |
| Antibiotic | Sulfadiazine | 68-35-9 | 6.65E-05 | 6.74E-05 | 6.74E-05 | 1.83E-04 | 1.85E-04 | 1.85E-04 | | | | | | |
| Antibiotic, | Sulfadimidine | 57-68-1 | 1.15E-04 | 1.17E-04 | 1.17E-04 | | | | | | | | | |
| Antibiotic | Sulfatroxazole | 23256-23-7 | | | | 4.47E-04 | 4.55E-04 | 4.55E-04 | | | | | | |
| Antibiotic | Sulfadoxine | 2447-57-6 | | | | 3.86E-05 | 3.96E-05 | 3.96E-05 | | | | | | |
| Antibiotic | Sulfamethizole | 144-82-1 | | | | | | | | | | | | |
| Antibiotic | Trimethoprim | 738-70-5 | | | | | | | 1.72E-05 | 1.88E-05 | 1.88E-05 | 5.16E-05 | 5.17E-05 | 5.17E-05 |
| Antibiotic | Tylosin | 1401-69-0 | | | | 6.37E-05 | 6.44E-05 | 6.44E-05 | | | | | | |
| Antibiotic | Erythromycin | 114-07-8 | | | | | | | 2.56E-07 | 2.57E-07 | 2.57E-07 | 7.68E-07 | 7.68E-07 | 7.68E-07 |
| Antibiotic | Tetracycline | 79-85-6 | | | | | | | 6.73E-05 | 6.94E-05 | 6.94E-05 | 2.02E-04 | 2.02E-04 | 2.02E-04 |
| Antibiotic | Salicylic acid | 63-36-5 | | | | | | | 2.58E-04 | 2.64E-04 | 2.64E-04 | 7.75E-04 | 7.75E-04 | 7.75E-04 |
| H2 receptor agonist | Cimetidine | 51481-61-9 | | | | | | | 1.62E-05 | 2.02E-05 | 2.02E-05 | 4.85E-05 | 4.89E-05 | 4.89E-05 |
| Hypertension | Amlodipine | 88150-42-9 | | | | | | | 3.98E-05 | 4.18E-05 | 4.18E-05 | 1.19E-04 | 1.20E-04 | 1.20E-04 |
| Hypertension | Furosemid | 54-31-9 | | | | | | | 3.47E-04 | 3.47E-04 | 3.47E-04 | 1.04E-03 | 1.04E-03 | 1.04E-03 |
| Analgesic | Paracetamol | 103-90-2 | | | | | | | 4.92E-04 | 4.92E-04 | 4.92E-04 | 1.48E-03 | 1.48E-03 | 1.48E-03 |
| Analgesic | Naproxen | 22204-53-1 | | | | | | | 1.42E-05 | 1.45E-05 | 1.45E-05 | 4.27E-05 | 4.27E-05 | 4.27E-05 |
| Analgesic, NSAID | Ibuprofen | 15687-27-1 | | | | | | | 1.84E-06 | 1.84E-06 | 1.84E-06 | 5.53E-06 | 5.53E-06 | 5.53E-06 |
| Analgesic, NSAID | Ketoprofen | 22071-15-4 | | | | | | | 9.46E-07 | 9.46E-07 | 9.46E-07 | 2.84E-06 | 2.84E-06 | 2.84E-06 |
| Analgesic, NSAID | Diclofenac | 15307-79-6 | | | | | | | 1.06E-06 | 1.06E-06 | 1.06E-06 | 3.17E-06 | 3.17E-06 | 3.17E-06 |
| Estrogen | Estrone (E1) | 53-16-7 | 5.34E-04 | 5.52E-04 | 5.52E-04 | 3.90E-04 | 4.03E-04 | 4.03E-04 | 1.13E-06 | 1.17E-06 | 1.17E-06 | 3.40E-06 | 3.40E-06 | 3.40E-06 |
| Estrogen | Estradiol (E2) | 50-28-2 | 1.07E-04 | 1.11E-04 | 1.11E-04 | 2.09E-04 | 2.16E-04 | 2.16E-04 | 3.71E-07 | 3.83E-07 | 3.83E-07 | 1.11E-06 | 1.11E-06 | 1.11E-06 |
| Estrogen | Estriol (E3) | 50-27-1 | | | | | | | 2.28E-07 | 2.33E-07 | 2.33E-07 | 6.85E-07 | 6.85E-07 | 6.85E-07 |
| Estrogen | Ethinyl-estradiol (EE2) | EPISUITE | | | | | | | 5.81E-08 | 5.82E-08 | 5.82E-08 | 1.74E-07 | 1.74E-07 | 1.74E-07 |

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# APPENDIX D

Overview of derived $PNEC_{soil}$ values, test systems, and applied assessment factors for all included compounds.

**Table 3. PART I.** $PNEC_{soil}$ (mg/kg dw) values for included metals and respective test systems and assessment factors (AF) used for deriving PNECs. EqP: calculation of $PNEC_{soil}$ from $PNEC_{aq}$ performed by reference; EqP from $PNEC_{aq}$: $PNEC_{soil}$ calculated from $PNEC_{aq}$ given by reference; EqP from QSAR: $PNEC_{soil}$ calculated from QSAR estimated aquatic toxicity; SSD: species sensitivity distributions.

| Compound | $PNEC_{soil}$ | Test system and AF | Reference |
|---|---|---|---|
| Antimony (Sb) | 37 | Chronic toxicity data available for three trophic levels. Lowest NOEC 370 mg/kg *Folsomia candida*, AF = 10 | (European Chemicals Agency, n.d.) |
| Aluminum (Al) | 1000 | Total aluminum is not correlated with toxicity for terrestrial plants or invertebrates. PNEC set to an arbitrary value of 1000 mg/kg | (European Chemicals Agency, n.d.) |
| Arsenic (As) | 0.5 | Chronic toxicity data available for three trophic levels. Lowest NOEC 5 mg/kg *Enchytraeus albidus*, AF = 10 | (European Chemicals Agency, n.d.) |
| Barium (Ba) | 207.7 | Baseline level of Ba in EU-top soils, AF = 2 | (European Chemicals Agency, n.d.) |
| Lead (Pb) | 166 | SSD, AF = 2 | (EURAS, 2008) |
| Boron (B) | 5.7 | SSD, AF = 2 | (European Chemicals Agency, n.d.) |
| Cadmium (Cd) | 1.15 | SSD, AF = 2 | (European Chemicals Bureau, 2007a) |
| Copper (Cu) | 65 | Not available | (European Chemicals Agency, n.d.) |
| Cobalt (Co) | 10.9 | SSD, AF = 2 | (European Chemicals Agency, n.d.) |
| Chromium (Cr III) | 62 | EqP from $PNEC_{aq}$. Chronic toxicity data available for three trophic levels. Lowest NOEC of 0.05 mg/L for *Oncorhynchus mykiss*, AF = 10 | (European Chemicals Bureau, 2005a) |
| Mercury (Hg) | 0.2 | Chronic toxicity data available for three trophic levels. NOEC for *Tenebrio molitor* 2 mg/kg, AF = 10 | (European Chemicals Agency, n.d.) |
| Molybdenum (Mo) | 9.9 | SSD, AF = 1 | (European Chemicals Agency, n.d.) |
| Nickel (Ni) | 29.9 | SSD, AF = 2 | (European Chemicals Agency, n.d.) |
| Selenium (Se) | 1000 | PNEC not available. Se toxicity is mainly observed in Se rich soil, such as India and China, and is not expected to occur in Danish soils. PNEC set to an arbitrary value of 1000 | (Garousi, 2017) |
| Silver (Ag) | 1.41 | SSD, AF = 3 | (European Chemicals Agency, n.d.) |
| Thallium (Tl) | 1 | Safe limit set by the Canadian EPA | (Canadian Counsil of Ministers of the Environment, 2018) |
| Tin (Sn) | 1000 | No toxicity observed for any aquatic species. PNEC set to an arbitrary value of 1000 mg/kg | (European Chemicals Agency, n.d.) |
| Uranium (U) | 50 | Chronic toxicity data available for three trophic levels. Suggested PNEC of 100 mg/kg, additional AF = 2 | (Sheppard et al., 2005) |
| Vanadium (V) | 7.2 | SSD, AF = 3 | (European Chemicals Agency, n.d.) |
| Zinc (Zn) | 26 | SSD, AF = 2 | (Commission;JRC, 2008) |

**Table 3. PART II**. $PNEC_{soil}$ (mg/kg dw) values for organic contaminants and respective test systems and assessment factors (AF) used for deriving PNECs. EqP: calculation of $PNEC_{soil}$ from $PNEC_{aq}$ performed by reference; EqP from $PNEC_{aq}$: $PNEC_{soil}$ calculated from $PNEC_{aq}$ given by reference; EqP from QSAR: $PNEC_{soil}$ calculated from QSAR estimated aquatic toxicity; SSD: species sensitivity distributions.

| Compound group | Compound name | $PNEC_{soil}$ | Test system and AF | Reference |
|---|---|---|---|---|
| AHC | Benzene | 0.14 | EqP from PNECaq | (Common Implementation Strategy for the Water Framework Directive, 2005) |
| AHC | Biphenyl | 0.53 | EqP | (European Chemicals Agency, n.d.) |
| AHC | Ethylbenzene | 0.88 | EqP | (European Chemicals Bureau, 2007b) |
| AHC | Naphthalene | 1.00 | NOECs or EC10s are available for three trophic levels. Lowest NOEC, *Folsomia candida*, AF = 10 | (European Union Risk Assessment Report, 2008) |
| AHC | p-tert-butyltoluene | 0.38 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| AHC | Toluene | 0.34 | Long-term studies on plants and earthworm. Lowest NOEC, earthworms, AF = 50 | (European Chemicals Bureau, 2003) |
| AHC | Xylene | 1.00 | EqP from PNECaq. It is proposed to apply the $PNEC_{aq}$ for benzene to represent the toxicity of xylene | (OSPAR Commission, 2014) |
| Chlorophenyls | 2,4-dichlorophenol | 0.29 | EqP from PNECaq, SSD, AF = 2 | (Jin et al., 2011) |
| Chlorophenyls | 2,4,6-trichlorophenol | 2.46 | EqP from PNECaq, SSD, AF = 2 | (Jin et al., 2012) |
| Chlorophenyls | 4-chloro-3-methylphenol | 6.40 | EqP | (European Chemicals Agency, n.d.) |
| Dioxins | HpCDD | 7.79E-05 | EqP, TEF = 0.01 | TEF |
| Dioxins | 23478-HxCDD | 6.04E-06 | EqP, TEF = 0.1 | TEF |
| Dioxins | 123678-HxCDD | 1.02E-05 | EqP, TEF = 0.1 | TEF |
| Dioxins | 12378-PeCDD | 1.38E-07 | EqP, TEF = 1 | TEF |
| Dioxins | 123789-HxCDD | 1.02E-05 | EqP, TEF = 0.1 | TEF |
| Dioxins | TCDD | 1.69E-07 | EqP from PNECaq, TEF of 1 | (European Commission, 2011) |
| Dioxins | OCDD | 3.35E-03 | EqP, TEF = 0.0003 | TEF |
| Furans | 1234678-HpCDF | 1.59E-04 | EqP, TEF = 0.01 | TEF |
| Furans | 123478-HxCDF | 7.03E-06 | EqP, TEF = 0.1 | TEF |
| Furans | 1234789-HpCDF | 1.59E-04 | EqP, TEF = 0.01 | TEF |
| Furans | 123678-HxCDF | 7.03E-06 | EqP, TEF = 0.1 | TEF |
| Furans | 12378-PeCDF | 1.02E-05 | EqP, TEF = 0.03 | TEF |
| Furans | 123789-HxCDF | 7.03E-06 | EqP, TEF = 0.1 | TEF |

**Table 3 PART II**. Continued

| | | | | |
|---|---|---|---|---|
| Furans | 234678-HxCDF | 7.03E-06 | EqP, TEF = 0.1 | TEF |
| Furans | 23478-PeCDF | 1.02E-06 | EqP, TEF = 0.3 | TEF |
| Furans | 2378-TCDF | 1.36E-06 | EqP, TEF = 0.1 | TEF |
| Furans | OCDF | 0.012 | EqP, TEF = 0.0003 | TEF |
| HAH | Chloroform | 18.40 | EqP | (Institut National de l'Environnement Industriel et des Risques (INERIS), 2007) |
| HAH | Dichloromethane | 0.33 | EqP | (European Chemicals Agency, n.d.) |
| HAH | Pentachloroethane | 0.15 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| HAH | Tetrachloroethylene | 0.011 | Long-term test on invertebrate, plant and bacteria. Lowest observed for nitrification. AF = 10 | (European Chemicals Bureau, 2005b) |
| HAH | Trichloroethylene | 0.23 | EqP | (European Chemicals Bureau, 2004a) |
| HAH | 1,4-dichlorobenzene | 0.10 | Short-term toxicity tests available for terrestrial plants and invertebrates. Lowest LC50, earthworms 96 mg/kg, AF = 1000 | (European Commission Joint Research Centre, 2004) |
| HAH | 2,5-dichloroanliline | 1.98E-03 | EqP from PNECaq | (Ministry of the Environment, 2005) |
| LAS | LAS | 4.60 | SSD, for terr. plants and invertebrates | (Jensen et al., 2001) |
| PAHs | 2-methylpyrene | 0.065 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| PAHs | 1-methylpyrene | 0.13 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| PAHs | 2-methylphenanthrene | 0.06 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| PAHs | Acenaphthene | 0.038 | Chronic toxicity data available for terrestrial plants and collembola. Lowest NOEC for *Lactuca sativa*, 1.9 mg/kg, AF=50 | (European Union Risk Assessment Report, 2008) |
| PAHs | Acenaphthylene | 0.29 | Only one EC10 is available, *Folsomia fimetaria*, AF = 100 (for argument of AF, reader is referred to original reference) | (European Union Risk Assessment Report, 2008) |
| PAHs | Antracene | 0.13 | Short- or long-term toxicity data available for annelida, macrophyta and collembola. Lowest EC10 *Folsomia fimetatria*, 6.3 mg/kg, AF = 50 | (European Union Risk Assessment Report, 2008) |
| PAHs | Benzo(a)anthracene | 0.079 | Chronic toxicity data available for annelids, crustaceans and collembola. Lowest EC10 *Oniscus asellus* 0.79 mg/kg, AF =10 | (European Union Risk Assessment Report, 2008) |
| PAHs | Benzo(a)fluorene | 0.04 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| PAHs | Benzo(a)pyrene | 0.053 | Chronic toxicity data available for three trophic levels. Lowest EC10, *Porcellio scaber* 0.53 mg/kg, AF = 10 | (European Union Risk Assessment Report, 2008) |
| PAHs | Benzo(ghi)perylene | 0.17 | EqP | (European Union Risk Assessment Report, 2008) |
| PAHs | Benzfluranthen b+j+k | 0.28 | EqP | (European Union Risk Assessment Report, 2008) |
| PAHs | Benzo(e)pyren | 0.31 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| PAHs | Chrysen/triphenyl | 0.55 | EqP | (European Union Risk Assessment Report, 2008) |
| PAHs | Dibenz(ah)anthracen | 0.054 | EqP | (European Union Risk Assessment Report, 2008) |

**Table 3. PART II.** Continued

| | | | | |
|---|---|---|---|---|
| PAHs | Dibenzothiophen | 0.13 | No data available. It is proposed to apply the PNEC for anthracene to represent the toxicity of dibenzothiophene | (OSPAR Commission, 2014) |
| PAHs | Dimethylphenanthren | 0.12 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| PAHs | Fluoranthen | 1.50 | Chronic toxicity data available for three trophic levels. Lowest EC10 nitrification 15 mg/kg, AF =10 | (European Union Risk Assessment Report, 2008) |
| PAHs | Fluoren | 1.00 | Chronic toxicity data available for three trophic levels. EC10 for *Folsomia fimetaria*. AF =10 | (European Union Risk Assessment Report, 2008) |
| PAHs | Indeno(1,2,3-cd)pyren | 0.13 | EqP | (European Union Risk Assessment Report, 2008) |
| PAHs | Perylene | NA | Excluded from assessment due to lack of information | |
| PAHs | Phenanthrene | 1.80 | Chronic toxicity data available for three trophic levels. EC10 for *Folsomia fimetaria* 18 mg/kg, AF = 10 | (European Union Risk Assessment Report, 2008) |
| PAHs | Pyrene | 1.00 | Chronic toxicity data available for three trophic levels. Lowest NOEC *Folsomia candida* reproduction 10mg/kg, AF = 10 | (European Union Risk Assessment Report, 2008) |
| PAHs | PAH SUM(19) | 0.038 | Estimated from average of single PAHs, AF = 10 | |
| PBDEs | PBDE 17 | 3.54E-03 | EqP from aquatic toxicity of PBDE 47 | |
| PBDEs | PBDE 28 | 3.54E-03 | EqP from aquatic toxicity of PBDE 47 | |
| PBDEs | PBDE 47 | 0.01 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| PBDEs | PBDE 49 | 0.01 | EqP from aquatic toxicity of PBDE 47 | |
| PBDEs | PBDE 66 | 0.01 | EqP from aquatic toxicity of PBDE 47 | |
| PBDEs | PBDE 85 | 0.01 | EqP from aquatic toxicity of PBDE 99 | |
| PBDEs | PBDE 99 | 2.74E-03 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| PBDEs | PBDE 100 | 0.01 | EqP from aquatic toxicity of PBDE 99 | |
| PBDEs | PBDE 153 | 0.08 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| PBDEs | PBDE 154 | 0.08 | EqP from aquatic toxicity of PBDE 153 | |
| PBDEs | PBDE 183 | 0.08 | EqP from aquatic toxicity of PBDE 153 | |
| PBDEs | PBDE 209 | 100 | Chronic toxicity data available for three trophic levels. No effects observed in the tested range, max conc. 1000mg/kg. AF = 10 | (Sverdrup et al., 2006) |
| PCBs | Arochlor | 1.00 | Chronic toxicity data available for three trophic levels. Lowest NOEC for PCBs in mixture on plant growth, AF =10 | (Jensen, 2012) |
| PCBs | Polychlorineret terphenyl | 0.14 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| PFSAs | PFOS | 0.2 | Toxicity data available for plants and earthworms. Lowest NOEC spring wheat 1 mg/kg, AF = 50 | (Jensen, 2012) |
| PFSAs | PFDA | 0.25 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| PFSAs | PFNA | 0.24 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| PFSAs | PFOSA | 0.029 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |

**Table 3. PART II**. Continued

| | | | | |
|---|---|---|---|---|
| PFSAs | PFOA | 281 | EqP from PNECaq | (Australian Government Department of Health National Industrial Chemicals Notification and Assessment Scheme, 2016a) |
| PFSAs | PFUnA | 0.38 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| Phenols | Bisphenol A | 3.70 | Chronic toxicity data available for three trophic levels. Lowest NOEC for plants 37 mg/kg, AF =10 | (Joint Research Centre - Institute for Health and Consumer Protection, 2010) |
| Phenols | NP2EO | 0.056 | EqP from PNECaq | (Australian Government Department of Health National Industrial Chemicals Notification and Assessment Scheme, 2016b) |
| Phenols | NP1EO | 0.10 | EqP from PNECaq | (Australian Government Department of Health National Industrial Chemicals Notification and Assessment Scheme, 2016b) |
| Phenols | Nonylphenol | 0.39 | Experimental dataset behind the PNEC was not available from the reference. EC10 for earthworm reproduction 3.44mg/kg, AF = 10 | (Brooke et al., 2005) |
| Phenols | Phenol | 0.14 | Acute toxicity data available. Lowest LC50 for *Eisenia foetida* 136 mg/kg, AF = 1000 | (European Chemicals Bureau, 2006) |
| Phosphate-triesters | Tricresyl-phosphate | 3.06E-03 | EqP | (Brooke, D N. Crookes, M J. Quarterman, P and Burns, 2009) |
| Phosphate-triesters | TCPP | 1.70 | Chronic toxicity data available for three trophic levels. Lowest NOEC for emergence of *Lactuca sativa* seedlings of 17mg/kg, AF = 10 | (European Chemicals Bureau, 2008a) |
| Phosphate-triesters | Tributhyl-phosphate | 0.64 | EqP | (European Chemicals Agency, n.d.) |
| Phosphate-triesters | Triphenyl-phosphate | 0.15 | EqP | (Brooke et al., 2009) |
| Phthalates | BBP | 5.84E-03 | EqP from PNECaq | (Petersen and Pedersen, 1998) |
| Phthalates | DOP | 1.83E-03 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| Phthalates | DHEA | 4.28E-03 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| Phthalates | DEHP | 13 | Chronic toxicity data available for three trophic levels. Lowest NOEC 130 mg/kg (the highest tested concentration), AF = 10 | (European Chemicals Bureau, 2008b) |
| Phthalates | DBP | 2 | Chronic toxicity data for *Zea mays*, NOEC 200mg/kg, AF = 100 | (European Chemicals Bureau, 2004b) |
| Phthalates | DEP | 0.17 | EqP from PNECaq | (Petersen and Pedersen, 1998) |
| Phthalates | DNP | 1000 | Chronic toxicity data available for three trophic levels. No effect in conc. From 1000-10.000 mg/kg, AF = 10 | (United States Consumer Product Safety Commission, 2010) |
| Phenols | Octylphenol | 6.69E-03 | EqP | (European Chemicals Bureau, 2006) |
| PCN | $PCN_{sum\ 35}$ | 0.03 | EqP | (Environment Canada and Health Canada, 2009) |
| PCA, short chained | PCA C10-C13 | 1.99 | EqP | (European Chemicals Bureau, 2004b) |
| PCA, med. chained | PCA C14-C17 | 12 | Chronic toxicity data available for three trophic levels. Lowest NOEC 120 mg/kg *Eisenia fetida*, AF = 10 | (European Chemicals Bureau, 2005c) |
| Biocide | Triclosan | 0.04 | SSD | (Amorim et al., 2010) |
| Biocide | Triclocarban | 0.018 | EqP from PNECaq | (USEPA, 2002) |

**Table 3. PART III**. $PNEC_{soil}$ (mg/kg dw) values for medical compounds and estrogens together with respective test systems and assessment factors (AF) used for deriving PNECs. EqP: calculation of $PNEC_{soil}$ from $PNEC_{aq}$ performed by reference; EqP from $PNEC_{aq}$: $PNEC_{soil}$ calculated from $PNEC_{aq}$ given by reference; EqP from QSAR: $PNEC_{soil}$ calculated from QSAR estimated aquatic toxicity; SSD: species sensitivity distributions.

| Compound group | Compound name | $PNEC_{soil}$ | Test system and AF | Reference |
|---|---|---|---|---|
| Antibiotic | Sulfadiazine | 0.019 | EqP from PNECaq | (Anskjær et al., 2013) |
| Antibiotic, | Sulfadimidine | 0.02 | Chronic or acute toxicity data available for plants and bacteria. Lowest NOEC 1 mg/kg plant growth, AF = 50 | (Jensen, 2012) |
| Antibiotic | Sulfatroxazole | 2.11E-03 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| Antibiotic | Sulfadoxine | 4.96E-03 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| Antibiotic | Sulfamethizole | 0.05 | EqP from PNECaq | (Mose Pedersen et al., 2007) |
| Antibiotic | Trimethoprim | 0.02 | Chronic or acute toxicity data available for plants and bacteria. Lowest NOEC 1 mg/kg plant growth, AF = 50 | (Jensen, 2012) |
| Antibiotic | Tylosin | 5.00 | Chronic or acute toxicity data available for three trophic levels. Lowest NOEC 50 mg/kg plant growth, AF = 10 | (Jensen, 2012) |
| Antibiotic | Erythromycin | 1.46E-04 | EqP from PNECaq | (Perazzolo et al., 2010) |
| Antibiotic | Tetracycline | 6.00 | Chronic or acute toxicity data available for plants and bacteria. Lowest LOEC 300 mg/kg bacterial respiration, AF = 50 | (Jensen, 2012) |
| Antibiotic | Salicylic acid | 0.17 | EpP | (European Chemicals Agency, n.d.) |
| H2 receptor agonist | Cimetidine | 0.039 | EqP from PNECaq | (Lee et al., 2015) |
| Hypertension | Amlodipine | 0.022 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| Hypertension | Furosemid | 0.014 | EqP from QSAR est. PNECaq | (National Food Institute, 2018) |
| Analgesic | Paracetamol | 73.58 | EpP | (European Chemicals Agency, n.d.) |
| Analgesic | Naproxen | 0.03 | EqP from PNECaq | (AstraZeneca, 2015) |
| Analgesic, NSAID | Ibuprofen | 1.69 | Chronic or acute toxicity data available for invertebrates and plants. Lowest EC10 for *Folsomia candida* 169 mg/kg, AF = 100 | (Jensen, 2012) |
| Analgesic, NSAID | Ketoprofen | 0.045 | EqP from PNECaq | (Bonvin et al., 2011) |
| Analgesic, NSAID | Diclofenac | 0.066 | Acute toxicity data available for *Folsomia candida*. EC10 65.7, AF = 1000 | (Jensen, 2012) |
| Estrogen | Estrone (E1) | 2.51E-03 | EqP | (Martín et al., 2012) |
| Estrogen | Estradiol (E2) | 9.90E-04 | EqP | (Martín et al., 2012) |
| Estrogen | Estriol (E3) | 0.711 | EqP | (Martín et al., 2012) |
| Estrogen | Ethinylestradiol (EE2) | 1.69E-03 | EqP | (Martín et al., 2012) |

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# APPENDIX E

Overview of calculated PEC/PNEC values.

**Table 4. PART I.** Calculated PEC/PNEC values for metals in the four scenarios. PECinit, PEC10, and PEC100 = PEC 30-day average initially after 1st, 10th and 100th year of application.

| | Cattle slurry | | | Pig slurry | | | Sludge 30kgP/ha/y | | | Sludge 90kg/ha/3y | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Compound name** | **PECinit /PNEC** | **PEC10 /PNEC** | **PEC100 /PNEC** | **PECinit /PNEC** | **PEC10 /PNEC** | **PEC100 /PNEC** | **PECinit /PNEC** | **PEC10 /PNEC** | **PEC100 /PNEC** | **PECinit /PNEC** | **PEC10 /PNEC** | **PEC100 /PNEC** |
| Antimony (Sb) | | | | | | | <0.001 | <0.001 | 0.002 | <0.001 | <0.001 | 0.002 |
| Aluminum (Al) | 0.001 | 0.008 | 0.081 | <0.001 | 0.002 | 0.021 | | | | | | |
| Arsenic (As) | | | | | | | 0.004 | 0.036 | 0.270 | 0.011 | 0.033 | 0.270 |
| Barium (Ba) | | | | | | | <0.001 | 0.004 | 0.041 | 0.001 | 0.004 | 0.041 |
| Lead (Pb) | | | | | | | <0.001 | 0.001 | 0.007 | <0.001 | 0.001 | 0.007 |
| Boron (B) | | | | | | | 0.002 | 0.020 | 0.120 | 0.006 | 0.019 | 0.121 |
| Cadmium (Cd) | <0.001 | 0.004 | 0.039 | <0.001 | 0.002 | 0.020 | <0.001 | 0.003 | 0.028 | 0.001 | 0.003 | 0.028 |
| Copper (Cu) | 0.001 | 0.012 | 0.118 | 0.007 | 0.073 | 0.709 | <0.001 | 0.001 | 0.010 | <0.001 | 0.001 | 0.010 |
| Cobalt (Co) | | | | | | | <0.001 | 0.001 | 0.012 | <0.001 | 0.001 | 0.012 |
| Chromium (Cr) | | | | | | | 0.001 | 0.013 | 0.133 | 0.004 | 0.012 | 0.132 |
| Mercury (Hg) | | | | | | | 0.001 | 0.012 | 0.124 | 0.004 | 0.011 | 0.123 |
| Molybdenum (Mo) | | | | | | | <0.001 | 0.002 | 0.010 | 0.001 | 0.002 | 0.010 |
| Nickel (Ni) | <0.001 | 0.003 | 0.022 | <0.001 | 0.002 | 0.019 | <0.001 | 0.002 | 0.018 | 0.001 | 0.002 | 0.018 |
| Selenium (Se) | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Silver (Ag) | | | | | | | 0.001 | 0.007 | 0.072 | 0.002 | 0.007 | 0.071 |
| Thallium (Tl) | | | | | | | <0.001 | <0.001 | 0.004 | <0.001 | <0.001 | 0.004 |
| Tin (Sn) | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Uranium (U) | | | | | | | <0.001 | <0.001 | 0.004 | <0.001 | <0.001 | 0.004 |
| Vanadium (V) | | | | | | | 0.001 | 0.006 | 0.062 | 0.002 | 0.006 | 0.062 |
| Zinc (Zn) | 0.011 | 0.109 | 1.021 | 0.083 | 0.825 | 7.757 | 0.007 | 0.073 | 0.689 | 0.022 | 0.066 | 0.684 |
| **Metals SUM PEC/PNEC** | **0.014** | **0.135** | **1.281** | **0.086** | **0.859** | **8.106** | **0.022** | **0.184** | **1.608** | **0.056** | **0.167** | **1.599** |

**Table 4. PART II**. Calculated PEC/PNEC values for organic contaminants in the four scenarios. For compounds where $PNEC_{soil}$ is estimated from $PNEC_{aq}$ and for which logKow is above 5, the resulting PEC/PNEC has been subjected to an additional factor of 10 to include exposure from direct ingestion of bound compound. These compounds are marked with *. PECinit, PEC10, and PEC100 = PEC 30-day average initially after 1st, 10th and 100th year of application.

| | | Cattle slurry | | | Pig slurry | | | Sludge 30kgP/ha/y | | | Sludge 90kg/ha/3y | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Compound group** | **Compound name** | **PECinit /PNEC** | **PEC10 /PNEC** | **PEC100 /PNEC** | **PECinit /PNEC** | **PEC10/ PNEC** | **PEC100 /PNEC** | **PECinit /PNEC** | **PEC10 /PNEC** | **PEC100 /PNEC** | **PECinit /PNEC** | **PEC10 /PNEC** | **PEC100 /PNEC** |
| AHC | Benzene | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| AHC | Biphenyl | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| AHC | Ethylbenzene | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| AHC | Naphthalene | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| AHC | p-tert-butyltoluene* | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| AHC | Toluene | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| AHC | Xylene | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Chlorophenyl | 2,4-dichlorophenol | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Chlorophenyl | 2,4,6-trichlorophenol | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Chlorophenyl | 4-chloro-3-methylphenol | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Dioxins | HpCDD* | | | | | | | 0.003 | 0.006 | 0.006 | 0.009 | 0.010 | 0.010 |
| Dioxins | 123478-HxCDD* | | | | | | | 0.001 | 0.001 | 0.001 | 0.002 | 0.002 | 0.002 |
| Dioxins | 123678-HxCDD* | | | | | | | 0.001 | 0.003 | 0.003 | 0.004 | 0.005 | 0.005 |
| Dioxins | 12378-PeCDD* | | | | | | | 0.034 | 0.066 | 0.066 | 0.101 | 0.114 | 0.114 |
| Dioxins | 123789-HxCDD* | | | | | | | 0.001 | 0.002 | 0.002 | 0.002 | 0.003 | 0.003 |
| Dioxins | TCDD* | | | | | | | 0.006 | 0.012 | 0.012 | 0.019 | 0.021 | 0.021 |
| Dioxins | OCDD* | | | | | | | 0.001 | 0.001 | 0.001 | 0.002 | 0.002 | 0.002 |
| Furans | 1234678-HpCDF* | | | | | | | 0.001 | 0.002 | 0.002 | 0.004 | 0.004 | 0.004 |
| Furans | 123478-HxCDF* | | | | | | | 0.001 | 0.002 | 0.002 | 0.004 | 0.004 | 0.004 |
| Furans | 1234789-HpCDF* | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Furans | 123678-HxCDF* | | | | | | | 0.001 | 0.002 | 0.002 | 0.003 | 0.004 | 0.004 |
| Furans | 12378-PeCDF* | | | | | | | <0.001 | 0.001 | 0.001 | 0.001 | 0.002 | 0.002 |

**Table 4. PART II**. Continued

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Furans | 123789-HxCDF* | | | | | | | 0.001 | 0.001 | 0.001 | 0.002 | 0.002 | 0.002 |
| Furans | 234678-HxCDF* | | | | | | | 0.001 | 0.002 | 0.002 | 0.004 | 0.004 | 0.004 |
| Furans | 23478-PeCDF* | | | | | | | 0.008 | 0.016 | 0.016 | 0.025 | 0.028 | 0.028 |
| Furans | 2378-TCDF* | | | | | | | 0.005 | 0.009 | 0.009 | 0.014 | 0.016 | 0.016 |
| Furans | OCDF* | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| HAHs | Chloroform | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| HAHs | Dichloromethane | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| HAHs | Pentachloroethane | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| HAHs | Tetrachloroethylene | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| HAHs | Trichloroethylene | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| HAHs | 1.4-dichlorobenzene | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| HAHs | 2.5-dichloroanliline | | | | | | | 0.006 | 0.006 | 0.006 | 0.017 | 0.017 | 0.017 |
| LAS | Alkylbenzensulfonat | 0.003 | 0.003 | 0.003 | 0.001 | 0.001 | 0.001 | 0.029 | 0.029 | 0.029 | 0.087 | 0.087 | 0.087 |
| PAHs | 2-methylpyrene* | | | | | | | 0.001 | 0.001 | 0.001 | 0.004 | 0.004 | 0.004 |
| PAHs | 1-methylpyrene* | | | | | | | <0.000 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| PAHs | 2-methylphenanthrene | | | | | | | <0.001 | <0.001 | <0.001 | 0.001 | 0.001 | 0.001 |
| PAHs | Acenaphthene | | | | | | | <0.001 | <0.001 | <0.001 | 0.001 | 0.001 | 0.001 |
| PAHs | Acenaphthylene | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| PAHs | Antracene | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| PAHs | Benzo(a)anthracene | | | | | | | <0.001 | <0.001 | <0.001 | 0.001 | 0.001 | 0.001 |
| PAHs | Benzo(a)fluorene* | | | | | | | 0.006 | 0.006 | 0.006 | 0.018 | 0.018 | 0.018 |
| PAHs | Benzo(a)pyrene | | | | | | | 0.001 | 0.001 | 0.001 | 0.002 | 0.002 | 0.002 |
| PAHs | Benzo(ghi)perylene* | | | | | | | 0.002 | 0.002 | 0.002 | 0.006 | 0.006 | 0.006 |
| PAHs | Benzfluranthen b+j+k* | | | | | | | 0.002 | 0.003 | 0.003 | 0.007 | 0.007 | 0.007 |
| PAHs | Benzo(e)pyren* | | | | | | | 0.001 | 0.001 | 0.001 | 0.003 | 0.003 | 0.003 |

**Table 4. PART II.** Continued

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PAHs | Chrysen/triphenyl* | | | | | | | 0.001 | 0.001 | 0.001 | 0.003 | 0.003 | 0.003 |
| PAHs | Dibenz(ah)anthracen* | | | | | | | 0.001 | 0.001 | 0.001 | 0.003 | 0.003 | 0.003 |
| PAHs | Dibenzothiophen | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| PAHs | Dimethylphenanthren* | | | | | | | 0.001 | 0.001 | 0.001 | 0.002 | 0.002 | 0.002 |
| PAHs | Fluoranthen | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| PAHs | Fluoren | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| PAHs | Indeno(1.2.3-cd)pyren* | | | | | | | 0.002 | 0.002 | 0.002 | 0.006 | 0.006 | 0.006 |
| PAHs | Perylene | | | | | | | | | | | | |
| PAHs | Phenanthrene | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| PAHs | Pyrene | | | | | | | | | | | | |
| PAHs | PAH SUM(19)* | 0.251 | 0.261 | 0.261 | 0.054 | 0.056 | 0.056 | | | | | | |
| PBDEs | PBDE 17* | | | | | | | 0.002 | 0.003 | 0.003 | 0.007 | 0.007 | 0.007 |
| PBDEs | PBDE 28* | | | | | | | 0.001 | 0.001 | 0.001 | 0.004 | 0.004 | 0.004 |
| PBDEs | PBDE 47* | | | | | | | 0.006 | 0.013 | 0.013 | 0.019 | 0.022 | 0.022 |
| PBDEs | PBDE 49* | | | | | | | 0.001 | 0.001 | 0.001 | 0.002 | 0.002 | 0.002 |
| PBDEs | PBDE 66* | | | | | | | <0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| PBDEs | PBDE 85* | | | | | | | 0.001 | 0.002 | 0.002 | 0.002 | 0.003 | 0.003 |
| PBDEs | PBDE 99* | | | | | | | 0.028 | 0.033 | 0.033 | 0.085 | 0.097 | 0.097 |
| PBDEs | PBDE 100* | | | | | | | 0.002 | 0.002 | 0.002 | 0.006 | 0.007 | 0.007 |
| PBDEs | PBDE 153* | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| PBDEs | PBDE 154* | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| PBDEs | PBDE 183* | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| PBDEs | PBDE 209 | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| PCBs | Arochlor* | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| PCBs | Polychlorineret terphenyl* | | | | | | | 0.001 | 0.001 | 0.001 | 0.002 | 0.002 | 0.002 |
| PFSAs | PFOS* | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

**Table 4. PART II**. Continued

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PFSAs | PFDA* | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| PFSAs | PFNA* | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| PFSAs | PFOSA* | | | | | | | 0.001 | 0.003 | 0.003 | 0.004 | 0.004 | 0.004 |
| PFSAs | PFOA* | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| PFSAs | PFUnA* | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Phenols | Bisphenol A | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Phenols | NP2EO* | 0.236 | 0.236 | 0.236 | 0.074 | 0.074 | 0.074 | 0.116 | 0.116 | 0.160 | 0.347 | 0.347 | 0.347 |
| Phenols | NP1EO* | | | | | | | 0.035 | 0.035 | 0.035 | 0.104 | 0.104 | 0.104 |
| Phenols | Nonylphenoles | 0.005 | 0.005 | 0.005 | 0.003 | 0.003 | 0.003 | 0.004 | 0.004 | 0.004 | 0.012 | 0.012 | 0.012 |
| Phenols | Phenol | | | | | | | 0.026 | 0.026 | 0.026 | 0.078 | 0.078 | 0.078 |
| Phosphate-triesters | Tricresylphosphate | | | | | | | 0.066 | 0.069 | 0.069 | 0.199 | 0.199 | 0.199 |
| Phosphate-triesters | TCPP | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Phosphate-triesters | Tributhylphosphate | | | | | | | <0.001 | <0.001 | <0.001 | 0.001 | 0.001 | 0.001 |
| Phosphate-triesters | Triphenylphosphate | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Phthalates | BBP | | | | | | | 0.004 | 0.004 | 0.004 | 0.011 | 0.011 | 0.011 |
| Phthalates | DOP* | | | | | | | 1.575 | 1.575 | 1.575 | 4.724 | 4.724 | 4.724 |
| Phthalates | DEHA* | | | | | | | 0.146 | 0.146 | 0.146 | 0.439 | 0.439 | 0.439 |
| Phthalates | DEHP | | | | | | | <0.001 | <0.001 | <0.001 | 0.001 | 0.001 | 0.001 |
| Phthalates | DBP | 0.001 | 0.001 | 0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Phthalates | DEP | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Phthalates | DNP | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Phenols | Octylphenol* | | | | | | | 0.013 | 0.013 | 0.013 | 0.040 | 0.040 | 0.040 |
| PCN | PCN (SUM35) | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| PCA, short chained | PCA C10-C13 | | | | | | | 0.005 | 0.005 | 0.005 | 0.014 | 0.015 | 0.015 |
| PCA, medium chained | PCA C14-C17 | | | | | | | 0.034 | 0.039 | 0.039 | 0.103 | 0.103 | 0.103 |

**Table 4. PART II**. Continued

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Biocide | Triclosan | | | | | | | 0.063 | 0.072 | 0.072 | 0.189 | 0.190 | 0.190 |
| Biocide | Triclocarban | | | | | | | 0.663 | 0.754 | 0.754 | 1.990 | 1.993 | 1.993 |
| **Org. compounds** | **SUM PEC/PNEC** | **0.428** | **0.439** | **0.439** | **0.110** | **0.113** | **0.113** | **2.915** | **3.128** | **3.128** | **8.744** | **8.791** | **8.792** |

**Table 4. PART III**. Calculated PEC/PNEC values for medical compounds and estrogens in the four scenarios. For compounds where $PNEC_{soil}$ is estimated from $PNEC_{aq}$ and for which logKow is above 5. the resulting PEC/PNEC has been subjected to an additional factor of 10 to include exposure from direct ingestion of bound compound. These compounds are marked with *. PECinit. PEC10. and PEC100 = PEC 30-day average initially after 1st, 10th and 100th year of application.

| | | Cattle slurry | | | Pig slurry | | | Sludge 30kgP/ha/y | | | Sludge 90kg/ha/3y | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Compound group** | **Compound name** | **PECinit /PNEC** | **PEC10 /PNEC** | **PEC100 /PNEC** | **PECinit /PNEC** | **PEC10 /PNEC** | **PEC100 /PNEC** | **PECinit /PNEC** | **PEC10 /PNEC** | **PEC100 /PNEC** | **PECinit /PNEC** | **PEC10 /PNEC** | **PEC100 /PNEC** |
| Antibiotic | Sulfadiazine | 0.003 | 0.003 | 0.003 | 0.009 | 0.010 | 0.010 | | | | | | |
| Antibiotic. | Sulfadimidine | 0.006 | 0.006 | 0.006 | | | | | | | | | |
| Antibiotic | Sulfatroxazole | | | | 0.212 | 0.216 | 0.216 | | | | | | |
| Antibiotic | Sulfadoxine | | | | 0.008 | 0.008 | 0.008 | | | | | | |
| Antibiotic | Sulfamethizole | | | | | | | <0.001 | <0.001 | <0.001 | 0.001 | 0.001 | 0.001 |
| Antibiotic | Trimethoprim | | | | | | | 0.001 | 0.001 | 0.001 | 0.003 | 0.003 | 0.003 |
| Antibiotic | Tylosin | | | | <0.001 | <0.001 | <0.001 | | | | | | |
| Antibiotic | Erythromycin | | | | | | | 0.111 | 0.138 | 0.138 | 0.332 | 0.335 | 0.335 |
| Antibiotic | Tetracycline | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Antibiotic | Salicylic acid | | | | | | | 0.003 | 0.003 | 0.003 | 0.009 | 0.009 | 0.009 |
| H2 receptor agonist | Cimetidine | | | | | | | 0.007 | 0.007 | 0.007 | 0.020 | 0.020 | 0.020 |
| Hypertension | Amlodipine | | | | | | | 0.003 | 0.003 | 0.003 | 0.009 | 0.009 | 0.009 |
| Hypertension | Furosemid | | | | | | | 0.003 | 0.003 | 0.003 | 0.009 | 0.009 | 0.009 |
| Analgesic | Paracetamol | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Analgesic | Naproxen | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Analgesic. NSAID | Ibuprofen | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Analgesic. NSAID | Ketoprofen | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Analgesic. NSAID | Diclofenac | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **Medical compounds** | **SUM PEC/PNEC** | **0.009** | **0.009** | **0.009** | **0.229** | **0.233** | **0.233** | **0.128** | **0.156** | **0.156** | **0.383** | **0.386** | **0.386** |

**Table 4. PART III**. Continued

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Estrogen | Estrone (E1) | 0.213 | 0.220 | 0.220 | 0.155 | 0.161 | 0.161 | <0.001 | <0.001 | <0.001 | 0.001 | 0.001 | 0.001 |
| Estrogen | Estradiol (E2) | 0.108 | 0.112 | 0.112 | 0.211 | 0.218 | 0.218 | <0.001 | <0.001 | <0.001 | 0.001 | 0.001 | 0.001 |
| Estrogen | Estriol (E3) | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Estrogen | Ethinylestradiol (EE2) | | | | | | | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| **Estrogens** | **SUM PEC/PNEC** | **0.321** | **0.332** | **0.332** | **0.366** | **0.379** | **0.379** | **0.001** | **0.001** | **0.001** | **0.003** | **0.003** | **0.003** |