Environmental pollution from pharmaceutical manufacturing -effects on vertebrates and bacterial communities

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid Göteborgs universitet kommer att offentligen försvaras i hörsal Arvid Carlsson, Academicum, Medicinaregatan 3, Göteborg, fredagen den 4 oktober 2013, kl. 9:00

av

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Avhandlingen baseras på följande delarbeten:

Paper 1. **Pharmaceutical industry effluent diluted 1:500 affects global gene expression, cytochrome p450 1a activity, and plasma phosphate in fish**
Gunnarsson L, Kristiansson E, Rutgersson C, Sturve J, Fick J, Förlin L, Larsson DGJ.
Environ Tox Chem, 2009, 28: 2639–2647.

Paper 2. **Oral exposure to industrial effluent with exceptionally high levels of drugs does not indicate acute toxic effects in rats**
Rutgersson C, Gunnarsson L, Fick J, Kristiansson E, Larsson DGJ.
Environ Tox Chem, 2013, 32: 577–584.

Paper 3. **Pyrosequencing of antibiotic-contaminated river sediments reveals high levels of resistance and gene transfer elements**
Kristiansson E, Fick J, Janzon A, Grabic R, Rutgersson C, Weijdegård B, Söderström H, Larsson DGJ.
PLoS One, 2011, 6(2): e17038.

Paper 4. **Antibiotics and antibiotic resistance genes in Indian well water and soil contaminated by industrial pollution**
Rutgersson C, Fick J, Marathe N, Kristiansson E, Janzon A, Flach C-F, Larsson DGJ.
Manuscript

Paper 5. **Quinolone resistance (*qnr*) genes in the gut flora of people living in an antibiotic-contaminated environment**
Rutgersson C, Marathe N, Kristiansson E, Moore ERB, Angelin M, Johansson A, Shouche Y, Flach C-F, Larsson DGJ.
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Abstract

High levels of pharmaceuticals, including fluoroquinolones (FQs), have been detected in the effluent from an Indian waste water treatment plant serving bulk drug production intended for the global market. Responses from short-term effluent exposure were studied in fish and rats through explorative analyses of hepatic mRNA abundance, enzyme activities and blood chemistry parameters. Exposure of rainbow trout to 0.2% effluent for five days altered hepatic gene expression and increased Cyp1a activity as well as blood plasma phosphate and cholesterol. In contrast, no effects could be demonstrated in rats tube-fed with effluent. Thus, exposure to effluent from drug manufacturing affects aquatic wild-life. No toxic effects were observed in rats after short-term exposure but risks associated with higher doses of effluent or a longer exposure time cannot be excluded.

High concentrations of FQs were found in sediment from the Indian river receiving drug-contaminated effluent, while no FQs were detected in sediment sampled near a municipal Swedish waste water treatment plant. Metagenome sequencing showed that resistance genes for several classes of antibiotics as well as genetic mobility elements were enriched in Indian sediments compared to Swedish samples. Selected antibiotic resistance genes were studied with qPCR in well water and soil from Indian villages. FQs were detected in samples from villages located <3 km from waterways with documented drug contamination. No enrichment of quinolone resistance genes (*qnr)* were seen in FQ-contaminated well water or soil while differences over seasons were observed for *sul2*, a sulfonamide resistance gene, and *intI1*, a class 1 integrase. Also, *qnr* were analyzed in human fecal samples. Three *qnr* genes were prevalent in fecal samples from Indians living in FQ-polluted as well as in FQ-free villages. The same three genes were detected, but less commonly, in stool samples from a group of Swedish students.

In conclusion, these studies demonstrate that discharges from antibiotic production lead to promotion of resistance genes and mechanisms facilitating their mobility in highly contaminated aquatic environments. Additional studies are required to elucidate the consequences of lower antibiotic concentrations in well water and soil, and the risk for transfer of antibiotic resistance genes from environmental bacteria to human intestinal flora. Once established, antibiotic resistance can rapidly spread over an extensive geographical area. Despite current knowledge gaps, the toxicity to wildlife and potential detrimental consequences for human health call for immediate and collaborative actions to improve waste management from drug manufacturing.

Keywords; pharmaceutical manufacturing, environmental drug contamination, antibiotic resistance, fluoroquinolones

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