Proceedings KVIV Seminar ‘Pharmaceuticals in the Environment’ March 9, 2000, Brussels

# Examples of exposure assessment simulation for pharmaceuticals in river basins with the GREAT-ER 1.0 system

***Diederik Schowanek* and Simon Webb***

Procter & Gamble Eurocor, Temselaan 100, B-1853 Strombeek-Bever, Belgium
* corresponding author: schowanek.d@pg.com

## ABSTRACT

The GREAT-ER (Geo-referenced Regional Exposure Assessment Tool for European Rivers) system was developed and validated by ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals), as an accurate aquatic chemical exposure prediction tool for use within environmental risk assessment schemes. The GREAT-ER 1.0 software calculates the distribution of predicted environmental concentrations (PECs) of consumer chemicals in surface waters, for individual river stretches as well as for entire catchments. The system uses an ARC/INFO - ArcView (® ESRI) based Geographical Information System (GIS) for data storage and visualization, combined with simple mathematical models for prediction of chemical fate. Prediction of environmental PECs of pharmaceuticals used in the household with GREAT-ER 1.0 is illustrated for Ethinyl Oestradiol, Paracetamol, Aspirin, Dextropropoxyphene, Clofibrate and Oxytetracycline in the Aire river basin (UK). In comparison with other household chemicals the eventual transformation of pharmaceuticals in the human body needs to be incorporated in the emission estimation. The PECinitial of these pharmaceuticals in surface waters ranges from >1 μg/l (Oxytetracycline & Paracetamol) down to 0.14 ng/l (Ethinyl Oestradiol).

## KEYWORDS

Environmental Risk Assessment, Geographic Information Systems (GIS), GREAT-ER model, Pharmaceuticals, River Basins.

## INTRODUCTION

The assessment of whether a substance presents a risk to organisms in the environment is based on a comparison of the predicted environmental concentration (PEC) of the substance with its predicted no-effect concentration (PNEC) to organisms in ecosystems. This assessment can be performed for different compartments (e.g. air, water and soil) and on different spatial scales (local, regional, continental). The European Union legislation related to risk assessment is described in a number of EU Commission documents (Technical Guidance Documents supporting the Commission Directive on Risk Assessment of New Chemicals (EEC, 1993) and Commission Regulation on Risk Assessment of Existing Substances (1488/94/EEC) in support of Existing Substances Regulation (EEC, 1994), and is applied in the computerized calculation model EUSES (European Union System for the Evaluation of Substances, European Chemicals Bureau, 1997). Realism in risk assessment can be increased by incorporating spatial and temporal characteristics of the receiving environment in the models and underlying databases. This is the methodology adopted in GREAT-ER (Fig. 1).

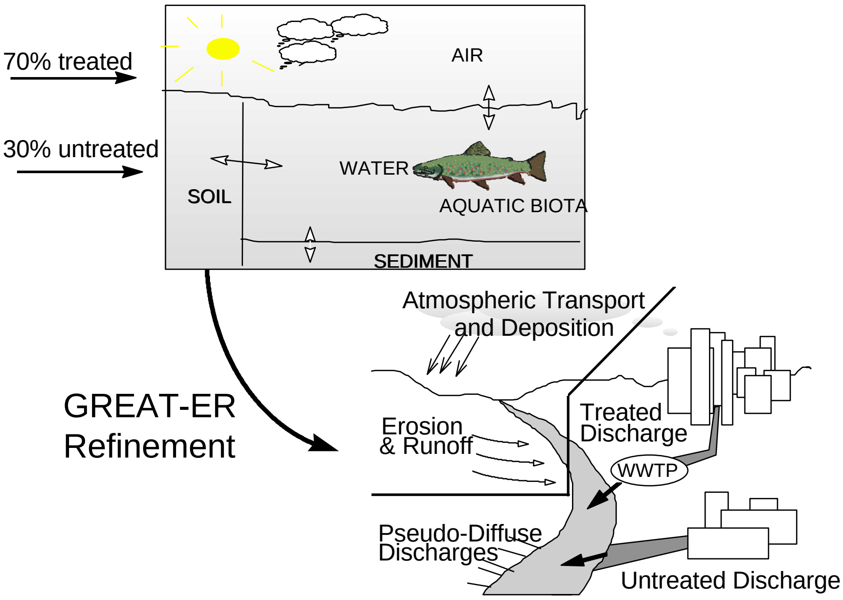


***Figure 1. Refinement of generic regional exposure models (EUSES) by taking actual discharge pathway, treatment and river flow data into account (GREAT-ER). (WWTP = wastewater treatment plant)***

## GREAT-ER CONCEPTS AND SYSTEM DEVELOPMENT

The GREAT-ER system has been approached in a modular way, as described in detail in Feijtel *et al.* (1997).

**GIS Data Manipulation:** In the data manipulation module, input data sourced from several databases and from the hydrology module (see below) are transformed into appropriate GIS formats (Wagner *et al.*, 1998).

**Hydrology:** The hydrology module combines several hydrological databases with a hydrological model. It provides the GREAT-ER system with the required river flow distributions, flow velocities and river characteristics. The Micro Low Flows model developed by the Institute of Hydrology to predict natural river flows at ungauged sites has been augmented with artificial influence data (abstractions, reservoirs, discharges) to give reliable predictions of flow distributions in the Yorkshire rivers (Young *et al.*, 1998).

**Waste Pathway and River Modelling:** This module is used for the prediction of chemical emission, of chemical removal/transformation during conveyance and treatment, and of chemical fate in rivers (Boeije *et al.*, 1997). Chemical fate in wastewater treatment plants and in rivers is described deterministically, with several levels of complexity being available to reflect the available information concerning both the chemical and the environment. For example, removal during sewage treatment can be either on a simple percentage removal basis, or alternatively it can be predicted using the SimpleTreat model (which is currently also used in EUSES (European Chemicals Bureau, 1997)).
On top of this, GREAT-ER applies a stochastic technique (i.e. Monte Carlo simulation), which allows most input parameters to be described in terms of a distribution (normal, log-normal, or uniform distributions can be specified). The Monte Carlo approach generally requires about 1000 runs for sufficient convergence to be obtained. Thus GREAT-ER can produce a statistical distribution of predicted environmental concentrations, as required for probabilistic risk assessment.

**End-User Desktop GIS:** In this module, access to and visualization of the databanks and model results is achieved, as well as the linking of the models with the data banks. The GIS databanks, the waste pathway models and the river models are integrated into one coherent simulation system. Such integration process results in an operational end-user system, which runs on a PC platform. The hydrological models and the ARC/INFO spatial data processing steps are not integrated into the end-user software system.

The user interface is the front-end between the user and the software system. It allows the selection of catchments, chemicals as well as the input of model and scenario parameters. The user interface also handles filtering and visualization of model results by the GIS. Avenue (® ESRI) has been used for the development of this interface in an ArcView (® ESRI) environment.

## OUTPUT OF GREAT-ER 1.0

GREAT-ER 1.0 offers a set of possibilities for analysis of the simulation results:

**Colour-coded River Maps:**
GREAT-ER's direct output provides predicted chemical concentrations linked to a river network, which are visualized as colour-coded digital GIS river maps (Fig. 4). To capture the spatial variability, the predicted concentrations are represented as quartiles of the distributions of all concentrations in the catchment. PECs can e.g. also be shown as absolute concentrations classes as defined by the user. The GIS analysis tools and colour-coded maps allow identification of any locations ('hot spots') within a region where site-specific PEC values may exceed the PNEC. General water quality maps may be overlain onto the simulation output to compare chemical presence with physico-chemistry- or biology-based water quality indices.

**Concentration profiles:**
Profiles of predicted concentrations through the studied catchment can be generated and exported. Such simulated profiles clearly illustrate chemical fate from a river's headwater down to its mouth, and can be used to directly compare model predictions with monitoring data, where available (Fig. 2).

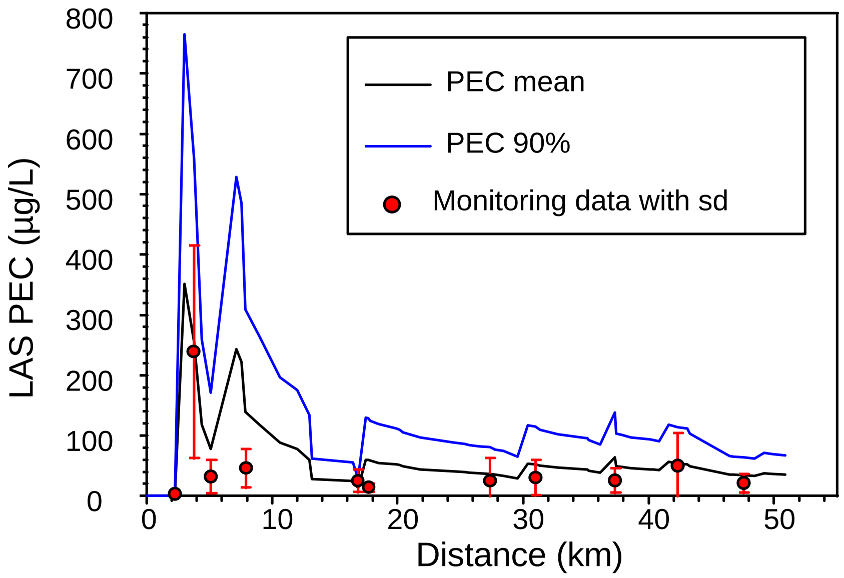


***Figure 2. Validation of GREAT-ER 1.0: example of the comparison between measured and simulated Linear Alkylbenzene Sulfonate (LAS - a detergent ingredient) concentrations for the Rother river, Yorkshire, UK.***

**Aggregated PECs:**
Geo-referenced model results can be aggregated to obtain a spatially averaged PEC (Fig. 3), which is representative of the river basin under study *(Boeije et al., 2000)*. GREAT-ER can generate a $\mathbf{PEC_{initial}}$ which comes from the distribution of concentrations in the river stretch below each emission point source.

This can be considered a GIS-analogue of the 'PEC-local' concept used in the EU TGD. GREAT-ER can also generate a $\mathbf{PEC_{catchment}}$ by incorporating the concentration distributions in each river stretch in the catchment. This involves a weighting procedure which can be based on stretch flow increment, length or volume. This concept can be considered a GIS analogue of the EU TGD 'PEC regional'.

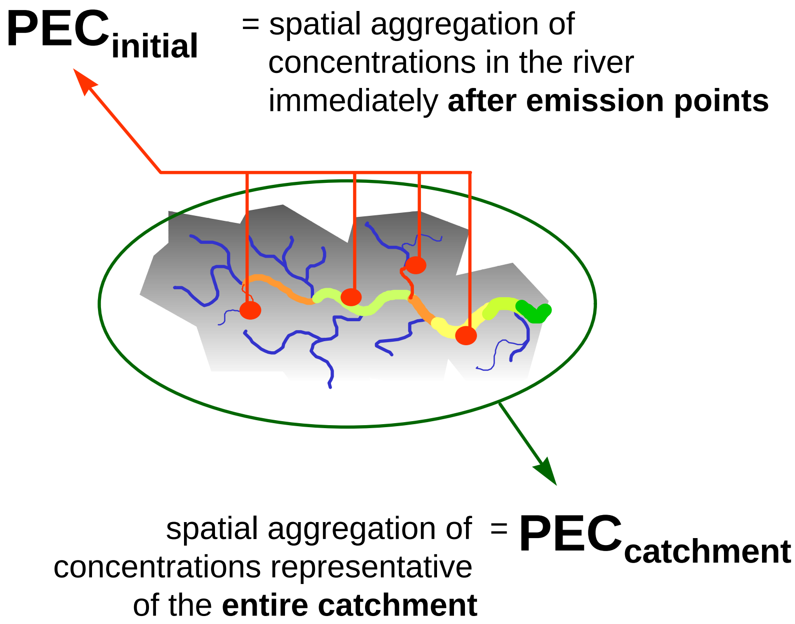


***Figure 3. Schematic illustration of the $PEC_{initial}$ and $PEC_{catchment}$ concepts, as developed within GREAT-ER for GIS-assisted regional risk assessment.***

## METHODS

***Case studies of human pharmaceuticals***

Six common human pharmaceuticals were selected to illustrate the functioning of GREAT-ER 1.0. For the purposes of comparison, a single river catchment was used (Aire, Yorkshire, UK). Details of the compounds and their usage, metabolism and environmental fate as employed in the GREAT-ER 1.0 simulations are given in Table 1.

An audit of compound usage data was commissioned from IMS and reflects all sales of products containing these compounds into retail pharmacies, dispensing general practitioners and hospital pharmacies in 1995. Data relating to the UK usage of OTC analgesics for the same period were obtained from the Paracetamol Information Centre and the European Aspirin Foundation. Therapeutic category is as detailed in the Merck Index (1989). Per capita use and "*worse-case*" sewage influent concentrations (i.e., assuming no human metabolism) are calculated on the basis of a UK population of 57.6 million and a specific water consumption of 259 litres/capita/day (WSA, 1994).

Effective post-metabolism exposure as factored into the simulations is based on a consideration of Dollery (1991). In some cases, such as Ethinyl Oestradiol or Clofibrate a significant proportion of an administered compound may be excreted unoxidised as conjugates in urine or bile/faeces. In these cases, it has been assumed that biological activity can be restored following microbially mediated biotransformation (i.e., de-conjugation). This has been reported for non-oestrogenic steroid metabolites e.g., Oestradiol-3-glucuronide (Panter *et al.*, 1999). Henschel *et al.* (1997) similarly speculates on the microbial reactivation of Paracetamol conjugates. In the case of Oxytetracycline, no metabolism occurs and 100% availability is assumed. For Dextropropoxyphene, extensive N-demethylation means that only about 1% is excreted from the body unchanged (although the major metabolite, norpropoxyphene is known to retain some pharmacological activity). For Aspirin, near complete metabolism to Salicylic Acid is assumed.

Removal during waste water treatment is based upon actual observations (i.e., Ternes, 1998; Ternes *et al.*, 1999; Stumpf *et al.*, 1999) or a "*worse-case*" assumption of 0% for Oxytetracycline and Dextropropoxyphene. Both of these compounds are non-readily biodegradable (Richardson & Bowron, 1985). River water dieaway rates employed are the default values from the EU Technical Guidance Document (CEC, 1996).

**Table 1. Overview of Compound Usage, Metabolism and Environmental Fate.**

| Parameter | Ethinyl Oestradiol | Paracetamol | Aspirin | Dextropro-poxyphene | Clofibrate | Oxytetracycline |
|---|---|---|---|---|---|---|
| Formula | $C_{20}H_{24}O_2$ | $C_8H_9NO_2$ | $C_9H_8O_4$ | $C_{22}H_{29}NO_2$ | $C_{10}H_{11}ClO_3$ | $C_{22}H_{24}N_2O_9$ |
| MW | 296.41 | 151.17 | 180.16 | 339.48 | 214.66 | 460.44 |
| CAS # | 57-63-6 | 103-90-2 | 50-78-2 | 469-62-5 | 882-09-7 | 79-57-2 |
| Therapeutic Category | Oestrogen | Analgesic; Anti-pyretic | Analgesic; Anti-pyretic; Anti-inflammatory | Narcotic Analgesic | Anti-hyperlipoproteinemic | Anti-bacterial |
| Use t/a (UK 1995) | 0.029 | 2,000 | 770 | 42.5 | 1.5 | 33.7 |
| Use (mg/cap/a) | 0.5 | 34,722 | 13,368 | 738 | 26 | 585 |
| "*Worse Case*" Influent (µg/l) | 0.005 | 367.3 | 141.4 | 7.81 | 0.28 | 6.19 |
| Excreted Post-Metabolism (%) | <1% unchanged 30% as conjugates [31%] | 2 - 5% unchanged 85% congugates [90%] | Assume 1% [1%] | <1% unchanged [1%] | 98% as conjugates of free acid [98%] | 100% unchanged [100%] |
| Biodegradation | Non-Readily Biodegradable (Schweinfurth *et al.*, 1996) | Readily Biodegradable [+acclimation] (Richardson & Bowron, 1985) | Readily Biodegradable (Richardson & Bowron, 1985) | Non-Readily Biodegradable (Richardson & Bowron, 1985) | Non-Readily Biodegradable (Richardson & Bowron, 1985) | Non-Readily Biodegradable [Tetracycline] (Richardson & Bowron, 1985) |
| Total removal via wastewater treatment (%) | 78% AS 64% TF (Ternes *et al.* 1999) | 98% AS (Ternes, 1998) | 81% AS (Ternes, 1998) | Assume 0% | 51% AS (Ternes 1998) 34% AS 15% TF (Stumpf *et al.* 1999) | Assume 0% |
| River Water Die-Away K ($d^{-1}$) | 0 | $4.7 * 10^{-2}$ | $4.7 * 10^{-2}$ | 0 | 0 | 0 |

## RESULTS

The results of the GREAT-ER 1.0 exposure simulations are presented in Table 2. Where available, some examples of actual measurements from surface waters are also provided for comparative purposes. PECinitial concentrations ranged from 0.14 ng/l for Ethinyl Oestradiol up to 10 µg/l for Paracetamol. PECcatchment concentrations were correspondingly lower (factor 0.55). Observations of actual concentrations of Ethinyl Oestradiol in UK sewage effluents are reported by Desbrow *et al.* (1996). They observed that Ethinyl Oestradiol was undetectable (<DL 0.2 ng/l) in more than half of the effluents sampled and where detectable was usually below 1 ng/l. Allowing for dilution, these observations are not inconsistent with the surface water PECs reported here. Williams *et al.* (1999) have also estimated likely Ethinyl Oestradiol concentrations in the Aire using the EXAMS model. Under conditions of low flow (i.e., summer) they predict concentrations of 0.15 ng/l and 0.10 ng/l at 1 km and 10 km downstream of an effluent discharge in a modelled stretch of the river. This is very similar to that predicted by GREAT-ER 1.0. It is interesting to note that Oxytetracycline was not detected in German surface waters (<DL 0.02 µg/l) by Hirsch *et al.* (1999). This contrasts with the predicted surface water PECs of approximately 1 µg/l reported here. One possible explanation for this may be that Oxytetracycline is photo-degradable in surface waters as has been reported for Tetracycline (Peterson *et al.* 1993). Other additional amelioration mechanisms ignored here include in-sewer biodegradation. Readily biodegradable substances such as Paracetamol are likely to be subject to a substantial degree of in-sewer biodegradation and as such the surface water PECs generated here are likely to be gross overestimates. This is borne out by the fact that Paracetamol was similarly not detected in German surface waters by Ternes (1998). Differences between the simulated PECs for the UK and reported concentrations of Clofibrate in German surface waters may reflect differing use patterns in the two countries (cf. 1.5 t/a in the UK with 16 t/a reported for Germany by Ternes, 1998). An example of the GIS output for a simulation is presented in Figure 4.

**Table 2. Calculation of PECinitial and PECcatchment (flow increment method) for 6 common pharmaceuticals.**

| Parameter | Ethinyl Oestradiol | Paracetamol | Aspirin | Dextropro-poxyphene | Clofibrate | Oxytetracycline |
|---|---|---|---|---|---|---|
| GREAT-ER PEC initial (µg/l) | 0.14 ng/l | 10.0 | 0.10 | 0.02 | 0.06 | 1.63 |
| GREAT-ER PEC catchment f.i. (µg/l) | 0.09 ng/l | 6.3 | 0.06 | 0.01 | 0.04 | 0.91 |
| MEC (µg/l) | <0.2 ng/l (Kalbfus, 1995) | Max. <0.15 (dl) (Ternes, 1998) | Med. <0.02 (dl) 90%-ile 0.16 Max. 0.34 (Ternes, 1998) | 1 (Richardson & Bowron, 1985) | Med. 0.066 90%-ile 0.21 Max. 0.55 Clofibric Acid (Ternes, 1998) | Med. <0.02 (dl) 90%-ile <0.02 (dl) Max. <0.02 (dl) (Hirsch *et al.*, 1999) |

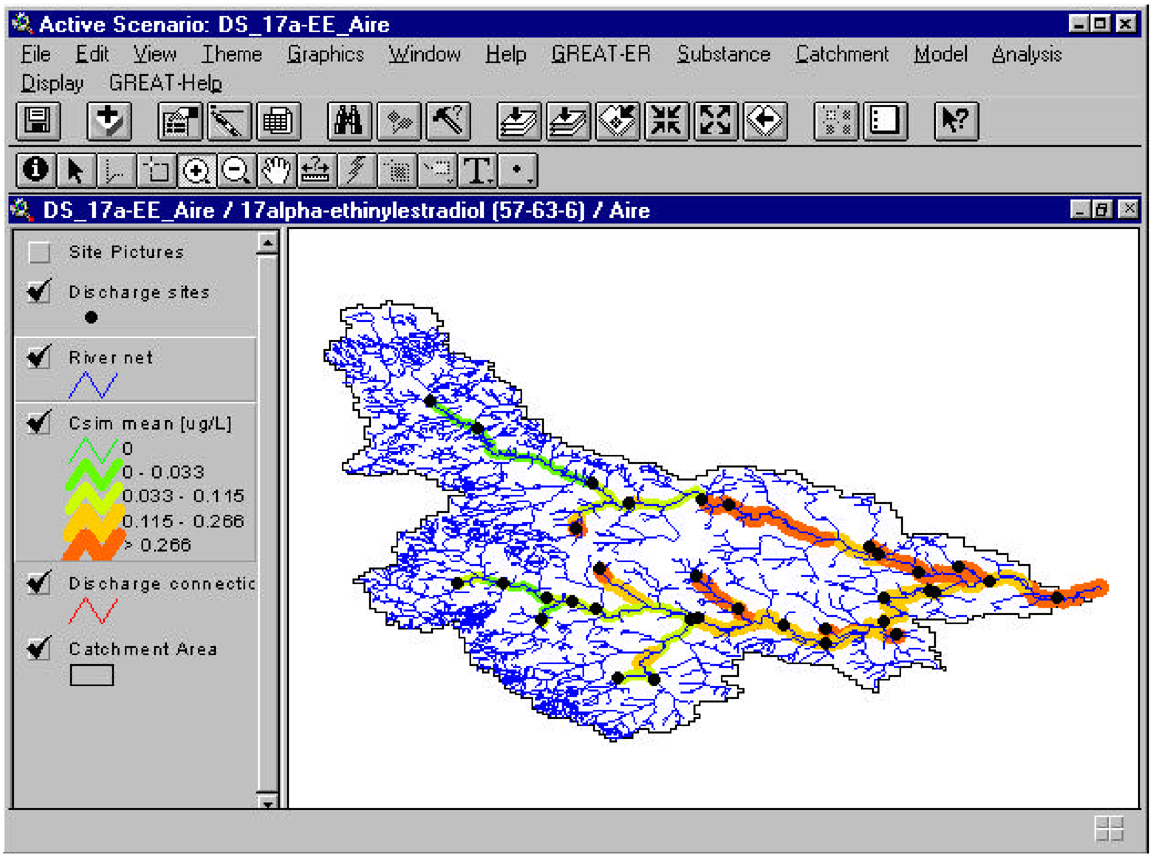


**Figure 4. GREAT-ER 1.0 user interface and colour coded GIS map with the simulation of Ethinyl Oestradiol in the Aire basin (Yorkshire, UK). [NB: units are ng/l - calculations were factored by 1,000 due to software considerations].**

PEC/PNEC ratios for the six compounds are presented in Table 3. The risk characterisation employs the PECinitial values from Table 2 and PNEC values derived by the application of an appropriate assessment factor to acute or chronic aquatic ecotoxicity data (CEC, 1996). For Ethinyl Oestradiol, Paracetamol and Oxytetracycline the PEC/PNEC ratios are greater than unity. In the case of Paracetamol, the incorporation of a consideration of in-sewer die-away and actual river water die-away data would undoubtedly improve the PEC. Paracetamol has not been detected in surface waters (Richardson & Bowron, 1985; Ternes, 1998). There is also scope to improve the PNEC by generating chronic effects data. For Oxytetracycline, it is likely that an incorporation of potential photodegradation (as discussed above) would have some impact upon the PEC. In addition, there is similarly scope for additional revision of the ecotoxicity database as the current PNEC is based on acute data. In the case of Ethinyl Oestradiol, no further revision of the ecotoxicity database is possible (with the exception of a mesocosm study). Furthermore, the observations of

Desbrow *et al.* (1996) support the simulated PEC values reported here. As such, claims of nominal environmental safety of Ethinyl Oestradiol in the Aire catchment are precluded.

**Table 3. Risk Characterisation Calculations (PEC/PNEC ratios) for 6 common pharmaceuticals**.

| Parameter | Ethinyl Oestradiol | Paracetamol | Aspirin | Dextropro-poxyphene | Clofibrate | Oxytetracycline |
|---|---|---|---|---|---|---|
| Ecotoxicity Data | 3 Chronic (Algae, Fish, *Daphnia*)<br><br>Länge *et al.* (1997); Kö pf (1995) | 3 Acute (Algae, Fish, *Daphnia*)<br><br>Henschel *et al.* (1997); Kühn *et al.* (1989) | 1 Acute (*Daphnia*)<br><br>Calleja *et al.* (1994) | 1 Acute (*Daphnia*)<br><br>Lilius *et al.* (1994) | 2 Chronic (Algae, *Daphnia*)<br><br>Kö pf (1995) | 3 Acute (Algae, Fish, Invertebrate)<br><br>Hughes (1973); Johnson (1976); Holten-Lutzhøft*et al.* (1998) |
| Lowest Endpoint (mg/l) | 1 ng/l | 9.2 | 168 | 14.6 | 10 µg/l | 0.23 |
| Assessment Factor | 10 | 1,000 | 1,000 | 1,000 | 50 | 1,000 |
| PNEC (µg/l) | 0.1 ng/l | 9.2 | 168 | 14.6 | 0.2 | 0.23 |
| GREAT-ER PEC initial (µg/l) | 0.14 ng/l | 10.0 | 0.10 | 0.02 | 0.06 | 1.63 |
| PEC/PNEC Ratio | 1.4 | 1.1 | <0.01 | <0.01 | 0.3 | 7.1 |

## CONCLUSIONS

- A preliminary estimation of the environmental concentrations of six pharmaceuticals was made by GREAT-ER 1.0 for the Aire basin. In first instance, the usage figures needed to be corrected for metabolism by the human body. After this correction, the pharmaceuticals can be treated as other down-the-drain domestic chemicals.

- There remains some degree of uncertainty around the removal in sewage treatment and in-stream die-away rates of most pharmaceuticals. Literature data were used for these simulations at tier 1 of the model, and 0% removal was assumed if no data were available. Generation of removal data or application of the SimpleTreat module in tier 2 of GREAT-ER 1.0 could further refine the assessment.

- Highest predicted concentrations in the Aire are found for Paracetamol (high per-capita usage, partial metabolism, high sewage treatment removal, surface water die-away) and the lowest for Ethinyl Oestradiol (low per-capita usage, partial metabolism, some sewage treatment removal, no surface water die-away). The PECcatchment for these chemicals is 0.55 x PECinitial.

- Based on these initial simulations, the next steps in the risk assessment process would be to refine the input data and GREAT-ER calculations, in particular for Ethinyl Oestradiol, Paracetamol and Oxytetracycline for which a PEC/PNEC ratio around or greater then unity are calculated.

## ACKNOWLEDGMENTS

The authors thank the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), the Environmental Risk Assessment Steering Management committee (ERASM) of the Association Internationale de la Savonnerie, de la Détergence et des Produits d'Entretien (A.I.S.E.) and the Comité Européen de Agents de Surface et Intermédiares Organiques (CESIO) and the UK Environment Agency for their financial and management support during the development of GREAT-ER. A copy of the CD-ROM and user manual can be obtained from ECETOC free of charge.

## REFERENCES

Boeije, G.M., Vanrolleghem, P. and Matthies, M. (1997). A geo-referenced aquatic exposure prediction methodology for 'down-the-drain' chemicals. Contribution to GREAT-ER # 3. *Wat. Sci. technol.* 36, 251-258.

Boeije, G.M., Wagner, J-O., Koorman, F., Vanrolleghem, P.A., Schowanek, D.R., and Feijtel, T.C.J. (2000) New PEC definitions for river basins applicable to GIS-based environmental exposure assessment. Contribution to GREAT-ER # 8. *Chemosphere*, in print.

Calleja, M.C., Personne, G. and Geladi, P. (1994). Comparative acute toxicity of the first 50 Multicentre Evaluation of *In Vitro* Cytotoxicity chemicals to aquatic non-vertebrates. *Arch. Environ. Contam. Toxicol.* 26, 69 - 78.

CEC (1996). *Technical Guidance Document in support of the Commission Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation (EEC) No. 1488/94 on risk assessment for existing substances*. European Chemicals Bureau, Ispra, Italy.

Desbrow, C., Routledge, E., Sheahan, D., Waldock, M. and Sumpter, J.P. 1996. "*The identification of oestrogenic substances in sewage treatment effluents*". Report of the Environment Agency: Bristol, U.K.

Dollery, C.T. [Ed.] (1991). *Therapeutic Drugs - Volume 1 & 2*. Churchill Livingstone, Edinburgh.

ECETOC (1994). *HAZCHEM, a mathematical model for use in risk assessment of substances. ECETOC Special Report No. 8* (1994). European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium.

EEC (1993). *Commission Directive 93/67/EEC of 20 July 1993 laying down the principles for assessment of risks to man and the environment of substances notified in accordance with Council Directive 67/458/EEC*, Off. J. of the European Communities L 227/9 (1993).

EEC (1994). *Commission Regulation of laying down the principles for the assessment of risks to man and the environment of existing substances in accordance with Council Regulation (EEC)* No 793/93/EEC (1994).

EUSES (1997). *European Uniform system for the evaluation of substances (EUSES), version 1.0*. European Chemical Bureau, Ispra, Italy.

Feijtel T.C.J., Boeije, G.M., Matthies, M., Young, A., Morris, G., Gandolfi, C., Hansen, B., Fox, K., Holt, M., Koch, V., Schroder, F.R., Cassani, G., Schowanek, D., Rosenblom, J. and Niessen, H. (1997). Development of a Geography-referenced Regional Exposure Assessment Tool for European Rivers - GREAT-ER. Contribution to GREAT-ER #1. *Chemosphere* 34, 2351-2374.

Henschel, K.P., Wenzel, A., Diderick, M. and Fliedner, A. (1997). Environmental hazard assessment of pharmaceuticals. *Regulatory Toxicology & Pharmacology* 25, 220-225.

Hirsch, R., Ternes, T., Haberer, K. and Kratz, K-L. (1999). Occurrence of antiobiotics in the aquatic environment. *Science Total Environment* 225, 109-118.

Holten-Lützhøft, H-C., Halling-Sorensen, B. and Jorgensen, S.E. (1998). Algal testing of antiobiotics applied in Danish fish farming. *Proc. 8th Annual Meeting of SETAC Europe, Bordeaux 1998.*

Hughes, J.S. (1973). *Acute Toxicity of Thirty Chemicals to Stripped Bass* (*Morone saxatilis*). Presented at the Western Association of State Game and Fish Commissioners in Salt Lake City, Utah July 1973.

Johnson, S.K. (1976). *Twenty-Four Hour Toxicity Tests of Six Chemicals to Mysis Larvae of Penaeus setiferus.* Texas A & M University Extension Disease Laboratory, Publication No. FDDL-S8.

Kalbfus, W. (1995). Belastung bayerisches Gewässer durch synthetische Östrogene. Vortag bei der 50. Fachtagung des Bayerisches Landesamt für Wasserwirtschaft: Stoffe mit endokriner Wirkung im Wasser (Abstract). [Effects in Bavarian watercourses through synthetic oestrogens. Presentation at the 50th Seminar of the Bavarian Association for Waters Supply: Substances with endocrine effects in water (Abstract)].

Köpf, W. (1995). Wirkung endokriner Stoffe in Biotests mit Wasserorganismen. Vortag bei der 50. Fachtagung des Bayerisches Landesamt für Wasserwirtschaft: Stoffe mit endokriner Wirkung im Wasser (Abstract). [Effects of endocrine substances in bioassays with aquatic organisms. Presentation at the 50th Seminar of the Bavarian Association for Waters Supply. Substances with endocrine effects in water (Abstract)]. Quoted in Römbke *et al.* (1995).

Kühn, R., Pattard., M., Pernak, K.D. and Winter, A. (1989). Results of the harmful effects of selected water pollutants (anilines, phenols, aliphatic compounds) to *Daphnia magna*. *Water Research* 23(4), 495-499.

Länge, R., Schweinfurth, H., Croudace, C., and Panther, G. (1997). Growth and reproduction of fathead minnow (*Pimephales promelas*) exposed to the synthetic steroid hormone Ethinylestradiol in a life cycle test (Abstract). *Proc. 7th Annual Meeting of SETAC Europe, Amsterdam 1997.*

Lilius, H., Isomaa, B. and Holmström, T. (1994). A comparison of the toxicity of 50 reference chemicals to freshly isolated rainbow trout hepatocytes and *Daphnia magna*. *Aquatic Toxicology* 30, 47-60.

Merck (1989). The Merck Index - *An Encyclopaedia of Chemicals, Drugs and Biologicals* (11th Edition) [Ed. S. Budavari]. Merck & Co. Inc., Rahway (NJ), USA.

Panter, G.H., Thompson, R.S., Beresford, N. and Sumpter, J.P. (1999). Transformation of a non-oestrogenic steroid metabolite to an oestrogenically active substance by minimal bacterial activity. *Chemosphere* 38(15), 3579 - 3596.

Peterson, S.M., Batley, G.E. and Scammell, M.S. (1993). Tetracycline in antifouling paints. *Marine Pollution Bulletin* 26(2), 96-100.

Richardson, M.L. and Bowron, J.M. (1995). The fate of pharmaceutical chemicals in the aquatic environment. *J. Pharm. Pharmacol.* 37, 1-12.

Schweinfurth, H., Länge, R., and Schneider, P.W. (1996). Environmental risk assessment in the pharmaceutical industry. *3rd Eurolab Symposium - Testing and Analysis for Industrial Competitiveness and Sustainability*", Berlin 5 - 7th June 1996.

Stumpf, M., Ternes, T.A., Wilken, R-D., Rodrigues, S.V. and Baumann, W. (1999). Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil. *Science Total Environment* 225, 134-141.

Ternes, T.A. (1998). Occurrence of drugs in German sewage treatment plants and rivers. *Chemosphere* 32(11), 3245 - 3260.

Ternes, T.A., Stumpf, M., Mueller, J., Haberer, K., Wilken, R-D. and Servos, M. (1999). Behaviour and occurrence of estrogens in municipal sewage treatment plants - I. Investigations in Germany, Canada and Brazil. *Science Total Environment* 225, 81-90.

Wagner, J-O., Koorman, F. and Matthies, M. (1998). GREAT-ER analysis tools and connectivity - exposure at a regional scale. *Proc. 8th Annual Meeting of SETAC Europe, Bordeaux 1998.*

Williams, R.J., Jürgens, M.D. & Johnson, A.C. (1999). Initial predictions of the concentrations and distribution of 17ß-Oestradiol, Oestrone and Ethinyl Oestradiol in 3 English rivers. *Wat. Res.* 33(7), 1663-1671.

WSA (1994). *Waterfacts '94*. (Ed. D. Burnell). Water Services Association, London.

Young, A.R., Gustard, A., Crocker, K.M. and Round, C.E. Hydrological models for use in regional and local exposure assessment methodologies. *Proc. 8th Annual SETAC Europe meeting, Bordeaux, France 1998*.