THE DEVELOPMENT OF HARMONISED TESTS TO BE USED IN THE EAS CONCERNING PRODUCTS IN CONTACT WITH DRINKING WATER

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In 1999 the Regulators Group on Construction Products in contact with Drinking water (RG-CPDW), based on the outcome of expert meetings held in 1999, decided that research on enhancement of microbial growth, cytotoxicity, GC-MS of non-target compounds and potential to form disinfectant by-products was needed before these assessments could become part of the European Acceptance Scheme (EAS) for CPDW. The JRC took the co-ordination to organise the project and a consortium of fifteen laboratories from ten Member States was formed:

NL Kiwa Water Research (financial co-ordinator)
EC DG JRC IES IWM (scientific co-ordinator)
A Österreichisches Forschungsinstitut für Chemie und Technik (ÖFI)
D DVGW-Technologiezentrum Wasser (TZW)
Umweltbundesamt (UBA)
DK Danmarks Tekniske Universitet (TUD)
Dansk Toksikologi Center (DTC)
E Universitat Autònoma de Barcelona (UAB)
F Centre de Recherche et de Controle des Eaux de Paris (CRECEP)
Laboratoire d'Hygiene et de Recherche en Santé Publique (LHRSP)
I Istituto Superiore di Sanità (ISS)
P Empresa Portuguesa das Águas Livres (EPAL)
S Sveriges Provnings- och Forskningsinstitut (SP)
UK Thames Water (TWUL)
WRc-NSF (WRc)

Enhancement of microbial growth

Growth of microorganisms can lead to consumer complaints of taste and odour. More importantly, the growth of pathogenic organisms such as Legionella can cause a risk to the

consumer's health. Three national test procedures are applied at present to test CPDW for the potential to support microbiological growth. The British Standard 6920 exposes the material in a semi-dynamic way by refreshing the water every 3-4 days and measures the dissolved oxygen depletion as a surrogate for microbial growth. The German DVGW W270 method exposes the material in a dynamic way and measures the volume of the biofilm that is formed on the surface of the materials. The Dutch Biofilm Production Potential (BPP) method exposes the material in a static way and measures adenosinetriphosphate (ATP) as an indication of the concentration of active biomass. The Dutch method can also measure the microorganisms growing in the water in addition to the surface. In 1999 experts agreed that:

- it was not practicable to produce a European test based on harmonisation of the three existing test procedures
- there was unawareness of any other test procedure that might be suitable for consideration
- an ATP-based test probably provides the best basis for assessing the potential to promote micro-biological growth

The objectives for research are the improvement of the existing ATP-based BPP assessment and the optimisation of the test conditions in order to obtain an acceptable level of information in a time-scale as short as possible and thus minimise the cost of testing.
The procedure starts with cleaning the test pieces of the product by flushing with non/de-chlorinated tap water, a stagnation of 24 h with test water and flushing again. The cleaned material is put in test water, spike with an inoculum and incubated at 25°C. Pieces of the material are removed from the incubation flask for microbial growth assessment at different exposure times. The surface to volume ratio is maintained in the incubation flask by removing test water. This test water may be used to determine the microbial growth in the water. The microorganisms are removed from the test piece in water by ultra-sonic vibration in several treatments. The combined water samples are used for the ATP determination.
The ATP analysis is the basis for the test method. Kiwa has optimised the ATP analysis and used it for more than 10 years. Detailed instructions are given to the other participants about the ATP method and about the procedure to remove biomass from solid surfaces of test materials. The quality of the ATP-test is tested in round robin tests with the participants in the course of the project.
Test water with a low growth potential (high degree of biological stability) is needed for the test. Each participant has tested different (non-)treated tap and artificial waters over a period of about 2 months in order to select their own test water with the lowest ATP concentration (<10 ng/l). The effect of addition of 1% of surface water inoculum and addition of P and N were also studied. The low growth potential of the test water ensures a high sensitivity of the test, as the effects of the feed water are eliminated, and ensures a wide applicability of the test throughout EU Member States.
Products in contact with drinking water may contain a large variety of biodegradable compounds. The physiological properties of the bacterial population in the test water should not limit the use of these compounds as substrates. The presence of a large variety of microorganisms in the test water may ensure this. The effects of different types of inocula, e.g. river water or extracts from sand filters, on the biomass production is tested with various types of test water, taking into account the variation in ATP content between prokaryotes and eukaryotes.
Static test conditions enable to test the biomass on the product and in the water, but may not reflect the conditions in practice. The effect of four scenarios of water replacement on biomass production, both as attached and suspended growth, is investigated in semi-batch tests: static, once and twice a week replacement of test water and replacement of test water every two weeks.

Based on the outcome of the investigations on the effects of water replacement scenarios, a second series of tests is conducted with a number of selected materials to assess the applicability (effects of the selected variables), and the reproducibility of the method. Also experiments are conducted with these products, simulating practical conditions to enable evaluation of the test results, e.g. by testing pipe segments. In these tests and experiments heterotrophic plate counts, which are used in routine monitoring of drinking water quality, are used in addition to ATP analysis. Laboratories apply media that are commonly applied for statutory monitoring in the respective countries. This way, a translation of these data to existing data is possible. Furthermore, this will aid the selection of media for the final test procedure. A number of selected materials, representing the range of material types, will also be with the existing methods (BS 6920, W270, BPP test) for interpretation of the data obtained in the newly designed test in relation to present pass-fail criteria connected with the existing methods.

Cytotoxicity and genotoxicity

Many studies have already shown there is no correlation between the presence of compounds detected by chemical analysis and cytotoxic effects in a sample. This is due to the fact that the compounds analysed by chemical methods cover only a fraction of all the compounds present in a sample and the compound detected are not necessarily toxic. Furthermore synergic toxicological effects cannot be tested by chemical analysis. Although chemical analysis is necessary, it is not sufficient for a toxicological evaluation of a product in contact with drinking water. Chemical analysis and cytotoxicological assessment gives complementary information.

Cytotoxicity tests only measure the potential metabolic effect of the compounds released by the materials, and do not allow conclusions on the potential mutagenetic effects of compounds released. Risks of the genome damages by molecules which are present in tap water or which are released by materials in contact with drinking water may be significant. This is why a genotoxicity test should go alongside the cytotoxicity one.

CPDW are assessed on cytotoxicological effects in two Member States. The French test (AFNOR XP P 41-250-3) is based on the assessment of the inhibition of RNA synthesis in HeLa S3 cells caused by the compounds present in the leachate and the British Standard 6920 looks at the inhibition of the growth of kidney cells in the leachate. The British test has a subjective endpoint. No suitable genotoxicity test is available yet. In 1999 experts agreed that:

- a cytotoxicity test is necessary to assess the risk of CPDW for consumers
- the assessment of cytotoxicity should be complementary to the GC-MS assessment for non-suspected compounds
- the existing French cytotoxicity test is considered the more appropriate
- an assessment of genotoxicity should go alongside the assessment of cytotoxicity and the choice of an appropriate test needs further investigations

According to this agreement the objectives for the research are the development of a harmonised assessment of cytotoxicity based on the French method and the proposal for a proper assessment of genotoxicity that will be complementary to the assessment of cytotoxicity.

The existing cytotoxicity and genotoxicity tests applied in the 15 Member States are reviewed. The review concerning cytotoxicity will summarise the existing practices, guidelines and regulations in order to determine the variables which could be applied to the French cytotoxicity test, which is considered to be the most appropriate one, to improve it or, if necessary, to make it more reproducible or robust. The review concerning genotoxicity will be carried out in the same way in order to determine if one of the presently available tests

could be adapted for the testing and approval of the materials in contact with drinking water, especially in sensitivity.
The leachate used in the cytotoxicity test is produced by using prEN 12873-1 "Influence of materials on water intended for human consumption – influence due to migration – Part 1: test method for non-metallic and non-cementitious factory made products". Growing medium is added to the leachate, a blank of pyrodistilled water and a positive control solution of potassium dichromate. The pH of the sample, i.e. leachate, blank or positive control, is adjusted to 7.2. Cleaned HeLa S3 cells are re-suspended in the sample and the sample is divided into three. The sub-samples are incubated at 37°C for 19 h. ^{3}H-uridine is added to the sub-samples after incubation and after different times the sample is dropped on pre-treated chromatography paper for the determination of ^{3}H-uridine incorporation. At the end the sub-sample is dropped on non-treated chromatography paper for the determination of the total radioactivity. The chromatography papers are washed and the radioactivity is measured. For every sub-sample the results are corrected for a total radioactivity of 100 CPM. The results of the three sub-samples are plotted in a radioactivity versus reaction time. The slope of the results of every sub-sample is calculated and averaged to get the mean for the slope of the sample. The average slope of a sample is expressed as a percentage of the average slope of the blank and this is a measure of the cytotoxicological effect.
Only three laboratories own the adequate equipment and will perform an inter-laboratory comparison exercise using the French procedure and leachates from two organic CPDW. From this exercise the method will be improved concerning e.g. S/V, effect of incubation time, and check of viability of cells before and after incubation. Next the improved method will be applied to selected organic, cementitious and metallic materials to four experimental conditions in order to determine the most appropriate cytotoxicity test.

Non-suspected compounds by GC-MS
The aim of a gas chromatography-mass spectrometry (GC-MS) assessment is to ensure that significant unsuspected chemicals, i.e. not specified in the formulation of the material, do not migrate into drinking water. At present only the UK and France routinely apply such GC-MS assessments. The main differences are that BS 6920-4 spikes the leachate with deuterated internal standards and extracts the leachate at pH 2 whereas AFNOR XP P 41-250-2 use non-labelled internal standards and extracts the leachate at pH 2 and 10 and combines the extracts.
In 1999 experts agreed that:

- these two national test methods should be used to form the basis of a harmonised GC-MS method able to detect and identify compounds at relatively low levels ($\mu g\ l^{-1}$)
- the research should investigate the usefulness of setting up of a centralised database that would include the GC-MS data on identified and unknown substances that are detected when the assessment is applied in routine use as part of the EAS. The availability of such a database for CPDW test-laboratories will permit easier identification of the substances involved and would contribute to make future GC-MS assessments of materials more cost effective.

The objectives for research are to merge the French and British method and to propose a design and operation of GC-MS database considering existing products.
In the first stage all participating laboratories, after circulation and discussion of procedures by correspondence, agree (at a kick-off meeting) the GC-MS procedure to be used. The model for this will be the UK BS 6920 part 4 procedure, since this is written in detail in standard format. The methodology will be modified with respect to the French procedure and any other aspects agreed by the participants. Where appropriate, the methodology will reflect existing CEN TC230 (Water Analysis) methodology.

All laboratories will take part in a study of a reference mixture of organic chemicals (Refmix 1) covering a range of boiling point and polarity (agreed at the kick-off meeting) to ensure no significant differences in instrumental GC-MS performance using the agreed GC-MS procedure.
The French and UK sample solvent extraction procedures will be compared to a combined, harmonised solvent extraction procedure. Using a standard reference mixture (Ref 2) of organic acids, neutrals and bases the UK and French participating laboratories will both study solvent extraction with dichloromethane at:
1. sample pH of 2
2. sample pH of deionised water
3. sample pH of 2 and 11 – i.e. combined extracts from pH 2 and 11

The alternative use of solid phase extraction (SPE) will be investigated. On the basis of existing information gathered from a variety of sources (such as EU-Pesticide project SMT4CT962142 Optimisation and evaluation of multi-residue methods for priority pesticides) a SPE phase will be selected for study and comparison with the preferred solvent extraction method. The Swedish and Dutch participating laboratories will carry out this work. Using the above reference mixture each would study:
1. SPE at pH 2 + 11 (combined)
2. Preferred solvent extraction

If the selected SPE phase is shown to produce results comparable to the solvent extraction procedure then it will be further tested. To ensure that the SPE procedure is not adversely influence by matrix effects (high organic background from the material or residual reagent, such as ascorbic acid dechlorinating agent) a study using leachates from GRP and epoxy resin will be carried out by the French and UK participating laboratories. The UK would study epoxy resin and France the GRP. Each would process aqueous leachates (spiked with the reference mixture) using the following procedures:
1. Preferred solvent extraction procedure
2. Preferred SPE procedure

Laboratory migration procedures will be carried out using chlorinated (1 mg l^{-1}) and non-chlorinated test waters.
In the final stage all participating laboratories will study aqueous leachates from epoxy resin, polyester, rubber and cement with organic additive, all leachates spiked with internal standards and extracted by the preferred procedure. These extracts will be prepared and distributed to all participating laboratories, and will relate to chlorinated and non-chlorinated test waters. The extracts will be examined under identical conditions. Depending on the results repeat studies of such extracts will be undertaken to achieve satisfactory comparisons.
To facilitate the use of the GC-MS assessment procedure developed in this work for materials in contact with drinking water, the most useful design and operation of a GC-MS database will be discussed and recommended. This study will consider the value of a database dedicated to assessment of such materials (be based on GC-MS data from approval testing) compared to existing databases.

Disinfectant by-products
Products used in public water distribution systems are often treated after installation or renovation with high levels of disinfectants, to prevent the risk of microbiological contamination. In case of severe bacterial contamination (Coliforms, Legionella, etc.),

plumbing systems are also subjected to stringent disinfection procedures. In both cases, the disinfectant is usually chlorine, but also hydrogen peroxide is sometimes used.
The disinfection treatment can affect the quality of materials and, in consequence, change the migration of substances from the products and have undesirable effects on health and hygiene of the consumers. To avoid the occurrence of such effects and the risk of leaching of disinfection by-products such as haloforms it is essential to include a simulation of disinfection treatment in the EAS for organic CPDW.
Although most Member States carry out such procedures in practise, France is the only country that has included a simulation of this procedure in its test methodology. How to build the assessment of the possible effects of disinfection on CPDW into harmonised standards is a major problem.
The objectives for research are to review the use of high levels of disinfectants applied to drinking water distribution systems and to develop a validated test method for the simulation of disinfection for approval of CPDW.
A review will summarise the details on the current disinfection practices in the 15 Members States:

- the range of disinfection conditions and the trend to use alternatives for high levels of chlorine
- If available, some microbiological contamination episodes in drinking water distribution systems, and if appropriate, specific disinfection procedures to be applied, the existing practices, guidelines and regulations applied for public and private installations participants in the 15 European Member States.

Comparisons of the disinfection practice (conditions, frequency, etc.) between the different Member States will be carried out. For example, incidents of microbial contamination are probably more frequent due to the higher temperatures in the Southern European countries in summer and Scandinavian countries are not in favour of using chlorine.
Chlorine is the most commonly used oxidant, but more recently, for safety and environmental reasons, there has been a tendency to use hydrogen peroxide to disinfect tanks. This process is applied and approved in some countries.
Representative CPDW (organic, cementitious and metallic materials) will be subjected to a range of disinfection conditions in order to assess their potential to form disinfection by-products. A range of chlorine content and contact times will be investigated. A limited study will be carried out for disinfection with hydrogen peroxide since the use of this oxidant is mentioned in some of the European countries for storage systems.
The existing (pr)ENs standards of interest for assessment of inertness vs. water quality will be applied in order to perform the relevant measurements in the migration waters after contact with materials:

- if submitted to high level of disinfection treatment
- if not submitted to such treatments.

This stage will start with investigations of organic materials, including one cementitious product with organic additive, because test standards are already available. Metallic materials will be studied later because a test standard has to be developed. All partners of this workpackage will participate in organoleptic measurements and migration assessment according to their capacity to perform the type of analysis. The complementarity of the specific competencies and of national economic interest will be considered. The materials to be studied will be shared in relation with their major uses within countries.
Six organic materials will be selected based on their frequent use in European water distribution systems and their sensitiveness to chlorination. The choice of CPDW will

represent the full agreement of all the participants of WP4 in co-operation with those of WP3. Most probably epoxy resin coating, PVC U pipe, PE HD, GRP pipe with polyester resin, rubber sealing and cementitious product with organic additive will be studied.
Samples of each material will be subjected to 8 different disinfection conditions and then to the migration procedure according to EN 1420 (odour, flavour) and prEN 12873-1 (TOC, AOX, individual substances):

- contact times: 6 and 24 hours
- 4 conditions: no disinfectant, 10 and 50 mg Cl_2 l^{-1}, and 300 mg H_2O_2 l^{-1}

If stage 1 reveals other disinfection practices of interest, they will be considered for investigation.
The relevant parameters that will be assessed in the migration waters are:

- odour and flavour, which the primary parameters of consumers complains (EN 1622)
- AOX and trihalomethanes, which are typical by-products of chlorination (EN 1485 and ISO 10301)
- TOC, which will give a global information on leaching of organic compounds (EN 1484)
- GC-MS analysis will permit identification and semi-quantification of other substances. In the case that the results of odour and flavour, AOX, TOC and trihalomethanes are sufficient to assess the influence of disinfectants the GC-MS analysis can be omitted. If not, GC-MS analyses will be co-ordinated with WP3.

A model will be developed for disinfection procedure(s) and recommendation will be made for the measurement of relevant parameters and disinfection procedure. If the results of the compounds that leach from the studied materials are not affected by the disinfectant procedure, this study will recommend the RG-CPDW not to include a disinfectant test in the EAS.