

TNO Report

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Subject : Determination of man-made chemicals in human blood

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Date:

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1. Introduction

Man-made chemicals are used as additives in consumer products such as carpets, curtains, toys and electronic equipment. As a result those chemicals can be found not only in the environment, but also in food items and in common house dust. This implies that there is a potential for human exposure. Since many of these compounds have a lipophilic nature they may be bioaccumulated, resulting in prolonged residence time in the human body.

Greenpeace Nordic has requested TNO to determine a number of such compounds in eight human blood samples. The chemicals considered in this study are; brominated flame retardants, phthalates, synthetic musk compounds, organochlorine pesticides, metals, triclosan and perfluorinated compounds.

2. Samples and chemical parameters

2.1 Samples

Blood samples were collected by Greenpeace Nordic using a standard serum tube without any additions. The samples were centrifuged after collection and frozen in an upright position so the serum and plasma remain separated. Samples were received by TNO on April 9, April 24 and April 28, 2006. Samples were kept frozen during transport. Each sample consisted of 5 to 7 tubes containing a total of approximately 40 ml of blood. The TNO code of the sample and the description of Greenpeace were as follows:

<i>TNO code :</i>	<i>Description:</i>
52006066-01	Sofie Lassen-Kahle
52006066-02	Niels Christian Meyer-Bubber
52006066-03	Mette Vibe Utzon
52006066-04	Klaus Bondam
52006066-05	Troels Klovedal
52006066-06	Eric Peitersen
52006066-07	Karen Haekkerup
52006066-08	Andrea Elisabeth Rudolph

2.2 Chemical parameters

The chemical parameters determined in this examination in human blood are:

- Brominated flame retardants
- Phthalates
- Artificial musk compounds
- Organochlorine pesticides
- Triclosan
- Perfluorinated compounds
- Metals

3. METHODS

3.1 Sample pre-treatment

Upon receipt the samples were allowed to thaw. Typically three tubes of each sample were used for the determination of brominated flame retardants, synthetic musk compounds and organochlorine pesticides. For these determinations the serum layer was transferred into PTFE-capped glass vials and stored at 4°C until further analysis. Two tubes were used for the determination of triclosan and perfluorinated compounds in the whole blood. One tube was used for the determination of the metals.

3.2 Sample analysis

All glassware used in the analyses is cleaned, rinsed with demi-water and baked in oven for 16 hours at 280°C prior to use. All solvents are distilled prior to use to achieve low blank results. The extraction of serum and whole blood is essentially the same and described below.

A serum or whole blood sample is weight into a clean glass 60 ml vial. Methanol, 0.1 M HCl and a set of internal standards (one or more for each group of chemicals) is added to the sample. The sample is extracted three times with a hexane-diethyl ether mixture and centrifuged after each extraction to separate the organic phase. The combined extracts are washed with a 1% KCl-solution and dried with anhydrous sodium sulphate.

3.2.1 Determination of triclosan and perfluorinated compounds

Whole blood extracts are concentrated to a small volume without further purification. All glass materials are rinsed with methanol and methanol was added to the extract before concentrating further to remove all hexane-diethyl ether residues. The methanol extract was transferred to and processed further in polycarbonate tubes to minimize losses of the perfluorinated compounds. The final extracts were analysed with liquid chromatography coupled with mass spectrometry (LC/MS) in the selected ion monitoring mode (SIM).

3.2.2 Determination of brominated flame retardants, phthalates, synthetic musk compounds and organochlorine pesticides.

The serum extract is concentrated to a small volume and purified using a florisil clean-up procedure. Separate fractions are collected for the brominated flame retardants, the organochlorine pesticides, and the phthalates and synthetic musk compounds. The purified extracts are concentrated to a small volume and an injection standard is added. The final extracts are analysed with gas chromatography coupled with mass spectrometry (GC/MS) in the selected ion monitoring mode (SIM).

3.2.3 Determination of the metals cadmium, lead and mercury

A whole blood sample is fully digested using ultra-pure nitric acid. After dilution of the acid extract to a standard volume, cadmium and lead are determined using inductively coupled plasma combined with mass spectrometry (ICP/MS). Mercury is determined using cold vapour atomic fluorescence spectrometry (CV/AFS).

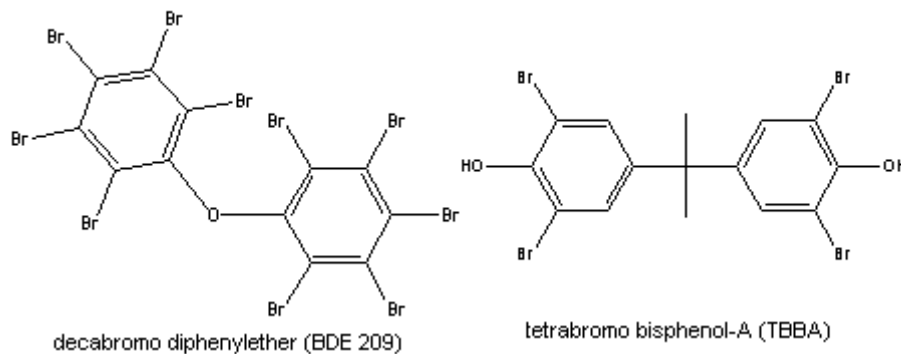
3.3 Identification, quantification and expression of results

The identification of analytes is based on correct retention times and/or qualifier ion ratios, compared to an external standard. The quantification was based on an external standard analysed together with the samples. The recovery of added internal standards was used to determine the performance of the analysis, but not to correct the results of the target compounds. The results are generally expressed in pg/g matrix for brominated flame retardants and ng/g matrix for the other parameters. Depending on the parameter the matrix is serum or whole blood.

4. RESULTS

4.1 Brominated flame retardants

Three types of brominated flame retardants are determined in this examination. The first are polybrominated diphenyl ethers (PBDE's), used as commercial mixtures with different degrees of bromination. Because of their structural similarity with PCB's, the individual PBDE's follow the same numbering as the PCB's. The second is hexabromocyclododecane (HBCD), a cyclo-aliphatic brominated chemical with the same application as PBDE's. The third is tetrabromobisphenol-A (TBBPA) mainly used as a flame retardant in epoxy polymers such as printed circuit boards in electronic equipment. The structure of BDE-209 (better known as deca-BDE) and TBBA is shown below.



The results for this study are presented in table 1, expressed in pg/g serum. BDE-47 was found in all samples, while BDE-28, -49, -100 and -153 were found in about half of the samples. The concentrations found in this study are comparable with earlier results for polybrominated diphenyl ethers in human blood. However, while the relative number of samples containing BDE-28 and -47 is higher than in a previous study, the relative number of samples containing BDE-153 and -99 is lower. BDE-209 and HBCD were found in none of the samples while tetrabromobisphenol-A was found in one sample in a concentration of 399 pg/g.

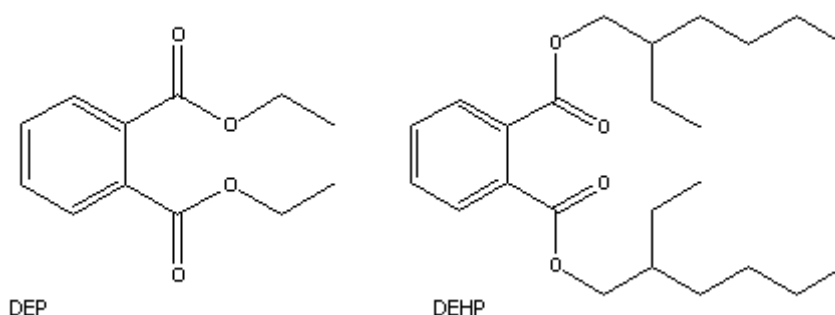
Table 1. Concentrations of brominated flame retardants found in blood serum in pg/g

Sample code: 5200	6066-01	6066-02	6066-03	6066-04	6066-05
Unit	pg/g serum	pg/g serum	pg/g serum	pg/g serum	pg/g serum
2,2',4-tribromo diphenylether (BDE 17)	<	<	<	<	<
2,4,4'-tribromo diphenylether (BDE 28)	11	10	11	19	<
2,2',4,4'-tetrabromo diphenyl ether (BDE 47)	22	10	14	21	42
2,2',4,5'-tetrabromo diphenylether (BDE 49)	6	7	<	<	<
2,2',3,4,4'-pentabromo diphenylether (BDE 85)	<	<	<	<	<
2,2',4,4',5-pentabromo diphenylether (BDE-99)	<	<	<	<	<
2,2',4,4',6-pentabromo diphenylether (BDE-100)	5	5	9	13	<
2,2',4,4',5,5'-hexabromo diphenylether (BDE-153)	13	15	18	31	<
2,2',4,4',5,6'-hexabromo diphenylether (BDE 154)	<	<	<	<	<
2,2',3,4,4',5',6-heptabromo diphenylether (BDE 183)	<	<	<	<	<
decabromo diphenylether (BDE 209)	<	<	<	<	<
hexabromocyclododecane (HBCD)	<	<	<	<	<
tetrabromobisphenol-A (TBBPA)	<	<	<	<	<

Sample code: 5200	6066-06	6066-07	6066-08	LOD
Unit	pg/g serum	pg/g serum	pg/g serum	pg/g serum
2,2',4-tribromo diphenylether (BDE 17)	<	<	<	< 1
2,4,4'-tribromo diphenylether (BDE 28)	<	<	30	< 1
2,2',4,4'-tetrabromo diphenyl ether (BDE 47)	18	26	47	< 2
2,2',4,5'-tetrabromo diphenylether (BDE 49)	<	<	8	< 1
2,2',3,4,4'-pentabromo diphenylether (BDE 85)	<	<	<	< 3
2,2',4,4',5-pentabromo diphenylether (BDE-99)	<	<	<	< 3
2,2',4,4',6-pentabromo diphenylether (BDE-100)	<	<	<	< 3
2,2',4,4',5,5'-hexabromo diphenylether (BDE-153)	<	<	132	< 5
2,2',4,4',5,6'-hexabromo diphenylether (BDE 154)	<	<		< 5
2,2',3,4,4',5',6-heptabromo diphenylether (BDE 183)	<	<	<	< 10
decabromo diphenylether (BDE 209)	<	<	<	< 100
hexabromocyclododecane (HBCD)	<	<	<	< 50
tetrabromobisphenol-A (TBBPA)	<	399	<	< 50

4.2 Phthalates

Phthalates are one of the most ubiquitous classes of compounds in our everyday environment. Phthalates are used as plasticizers to increase the flexibility of high molecular weight polymers and are also used as heat-transfer fluids and carries, and can be found in ink, paint, adhesives, pesticides and vinyl flooring. Consequently, the potential for human exposure is very high. Di-(2-ethylhexyl) phthalate (DEHP) accounts for a quarter of the plasticizers produced but is nowadays gradually replaced by iso-alkyl phthalate mixtures like di-isononyl phthalate (DINP). The chemical structure of di-ethyl phthalate (DEP) and DEHP is shown below.



The results for the phthalates are presented in table 2. Note that the results are expressed in ng/g serum. Surprisingly DEHP, one of the most common phthalates, was found in only three of the eight samples, however, with a high concentration in one of those samples, 6066-02. Sample 6066-03 did not contain any phthalates at all. The concentrations of the other phthalates are found in this study are comparable with previous results for phthalates in human blood. The iso-alkyl phthalate DIDP and dicyclohexyl phthalate DHCP were not found in the samples.

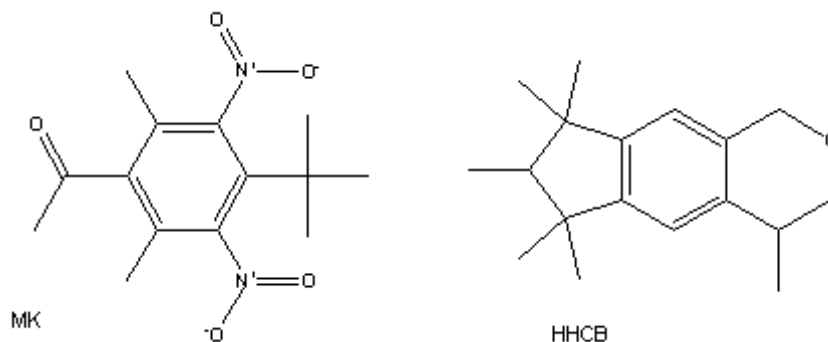
Table 2. Concentrations of phthalates in blood serum in ng/g

Sample code: 5200	6066-01	6066-02	6066-03	6066-04	6066-05
Unit	ng/g serum	ng/g serum	ng/g serum	ng/g serum	ng/g serum
di-methyl phthalate (DMP)	<	<	<	3.9	<
di-ethyl phthalate (DEP)	2.1	13	<	1.3	17
di-isobutyl phthalate (DIBP)	2.9	<	<	<	9.3
di-butyl phthalate (DBP)	<	<	<	124	<
butylbenzyl phthalate (BBP)	<	<	<	<	<
di-cyclohexyl phthalate (DCHP)	<	<	<	<	<
di-(2-ethylhexyl) phthalate (DEHP)	<	1614	<	<	<
di-isononyl phthalate (DINP)	<	<	<	<	<
di-isodecyl phthalate (DIDP)	<	<	<	<	<

Sample code: 5200	6066-06	6066-07	6066-08	LOD
Unit	ng/g serum	ng/g serum	ng/g serum	ng/g serum
di-methyl phthalate (DMP)	<	<	<	< 1
di-ethyl phthalate (DEP)	11	2.8	6.8	< 1
di-isobutyl phthalate (DIBP)	<	<	6.0	< 1
di-butyl phthalate (DBP)	<	<	16	< 10
butylbenzyl phthalate (BBP)	22	<	6.7	< 1
di-cyclohexyl phthalate (DCHP)	<	<	<	< 1
di-(2-ethylhexyl) phthalate (DEHP)	26	60	69	< 10
di-isononyl phthalate (DINP)	69	43	<	< 10
di-isodecyl phthalate (DIDP)	<	<	23	< 10

4.3 Artificial musks

Originally, musk is a male sexual scent signal but the increasing demand resulted in the production of artificial musk compounds. The most well known artificial musk are nitro musks like musk xylene (MX) and musk ketone (MK) that are nowadays replaced by polycyclic musks like tonalide (AHTN) and galaxolide (HHCB). Artificial musks are used as additives for perfumes, in detergents and soaps, in body lotions and deodorizers. The structure of MK and AHTN is presented below.



The results for the artificial musks are presented in table 3. Note that the results are in ng/g serum. As expected from earlier studies the polycyclic musks HHCB and AHTN are found in all and about half of the samples. Again the concentrations are comparable with earlier findings in human blood. Apart from HHCB and AHTN, ATTI was found in one of the samples. The older nitro-musks were found in none of the samples.

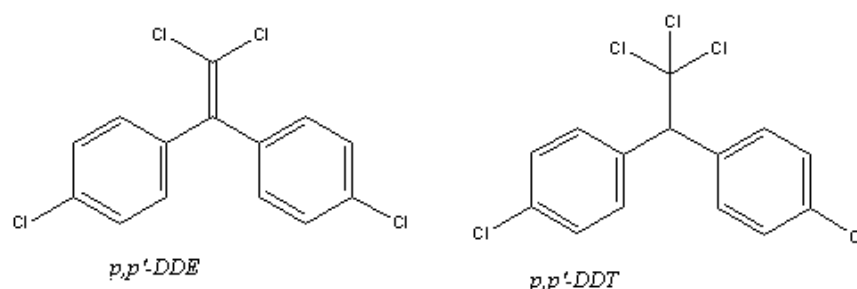
Table 3. Concentrations of artificial musks in blood serum in ng/g

Sample code: 5200	6066-01	6066-02	6066-03	6066-04	6066-05
Unit	ng/g serum	ng/g serum	ng/g serum	ng/g serum	ng/g serum
cashmeron (DPMI)	<	<	<	<	<
celestolide (ADBI)	<	<	<	<	<
galaxolide (HHCB)	0.66	1.9	0.15	0.71	0.41
tonalide (AHTN)	0.16	0.19	<	<	0.36
traseolide (ATTI)	<	<	<	<	<
musk ketone (MK)	<	<	<	<	<
musk xylene (MX)	<	<	<	<	<

Sample code: 5200	6066-06	6066-07	6066-08	LOD
Unit	ng/g serum	ng/g serum	ng/g serum	ng/g serum
cashmeron (DPMI)	<	<	<	< 0.1
celestolide (ADBI)	<	<	<	< 0.1
galaxolide (HHCB)	0.23	0.33	1.3	< 0.1
tonalide (AHTN)	0.68	0.28	0.17	< 0.1
traseolide (ATTI)	0.14	<	<	< 0.1
musk ketone (MK)	<	<	<	< 0.1
musk xylene (MX)	<	<	<	< 0.1

4.4 Organochlorine pesticides

In the past, organochlorine pesticides such as DDT were widely used on a global basis. Although their manufacture and application are now largely prohibited or restricted in industrialized western countries, they can still be found in environmental and biological matrices due to their persistence. In previous studies on human blood, ppDDE and ppDDT were found in most samples while opDDD, opDDE, opDDT and ppDDD were found in only a few samples. The ppDDE concentrations are often one factor higher than those of ppDDT, probably an indication that exposure to the DDT pesticide was either through indirect routes (e.g. through the diet) or some time in the past. Hexachlorobenzene is also commonly found in human blood. The structures of ppDDT and its breakdown product ppDDE are given below.



The results for a number of organochlorine pesticides are summarized in table 4. Note that the results are in ng/g serum. As expected, hexachlorobenzene, *o,p'*-DDE and *p,p'*-DDE were found in all samples with *p,p'*-DDE in the highest concentrations. Pentachlorobenzene and, *o,p'*-DDD and *o,p'*-DDT were found in one of the eight samples. In this study *p,p'*-DDT was not found in any of the samples while in an earlier study for Greenpeace *p,p'*-DDT was found in most samples.

Table 4. Concentrations of organochlorine pesticides in blood serum in ng/g

Sample code: 5200	6066-01	6066-02	6066-03	6066-04	6066-05
Unit	ng/g serum	ng/g serum	ng/g serum	ng/g serum	ng/g serum
pentachlorobenzene	<	<	<	<	<
hexachlorobenzene	0.09	0.11	0.11	0.19	0.20
<i>o,p'</i> -DDE	0.07	0.18	0.11	0.24	0.25
<i>p,p'</i> -DDE	0.37	0.26	0.82	1.1	1.7
<i>o,p'</i> -DDD	<	<	<	<	<
<i>p,p'</i> -DDD	<	<	<	<	<
<i>o,p'</i> -DDT	0.58	<	<	<	<
<i>p,p'</i> -DDT	<	<	<	<	<

Sample code: 5200	6066-06	6066-07	6066-08	LOD
Unit	ng/g serum	ng/g serum	ng/g serum	ng/g serum
pentachlorobenzene	0.05	<	<	< 0.01
hexachlorobenzene	0.27	0.12	0.01	< 0.01
<i>o,p'</i> -DDE	0.14	0.18	0.01	< 0.01
<i>p,p'</i> -DDE	1.2	0.28	0.08	< 0.01
<i>o,p'</i> -DDD	<	0.14	<	< 0.01
<i>p,p'</i> -DDD	<	<	<	< 0.01
<i>o,p'</i> -DDT	<	<	<	< 0.01
<i>p,p'</i> -DDT	<	<	<	< 0.01

4.5 Triclosan

Triclosan, also known as 5-chloro-2-(2,4-dichlorophenoxy)phenol, is a well known, and widely used, antibacterial and antimicrobial agent. Due to its effectiveness against bacteria that cause spoilage, odour and infection, triclosan has been incorporated into many common consumer products including toothpaste, deodorants, cosmetics, textiles, toys and antibacterial soaps and detergents. The increasing use of these products over the last 30 years has invariably led to triclosan, and its breakdown product methyltriclosan, being found in the environment. Because of its potential for bioconcentration triclosan may also be present in humans as indicated by two studies in Sweden that detected triclosan in human blood plasma and in human breast milk. The structure of TCS is shown below.

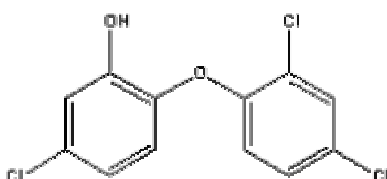


Table 5 shows a summary of results for triclosan expressed in ng/g whole blood. Triclosan was found in none of the samples in concentrations above the detection limit (LOD). This is different from the results of an earlier study for Greenpeace where triclosan was found in about half of the serum samples in concentrations ranging from 0.1 to 1.3 ng/g serum.

Table 5. Concentrations of triclosan in whole blood in ng/g

Sample code: 5200	6066-01	6066-02	6066-03	6066-04	6066-05
Unit	ng/g blood	ng/g blood	ng/g blood	ng/g blood	ng/g blood
triclosan	<	<	<	<	<

Sample code: 5200	6066-06	6066-07	6066-08	LOD
Unit	ng/g blood	ng/g blood	ng/g blood	ng/g blood
triclosan	<	<	<	< 0.2

4.6 Perfluorinated compounds

Perfluorinated compounds (PFCs) are synthetic compounds characterised by an alkyl chain in which the hydrogen atoms are completely replaced by fluorine atoms. PFCs are heat stable, very resistant to degradation and environmental breakdown and have an amphiphilic nature (they repel water as well as oil). Because of these properties PFCs are used a myriad of applications, such as non-stick pans and

stain/water repelling coatings for clothing, furniture and paper. The main PFCs detected in environmental samples are PFOS and PFOA. In addition to PFOS and PFOA other PFCs such as perfluorohexane sulfonate (PFHxS), perfluorononanoic acid (PFNA) and perfluorooctane sulphonamide (PFOSA) have been found.

The results for the PFCs are reported in table 6. In general low concentrations of PFOS and PFOSA were found in most of the samples. The identification of PFOA was hindered in the samples 6066-01 to 6066-04 due to an unknown interference. In the remaining samples PFOA was identified in concentrations up to 2.3 ng/g whole blood.

Table 6. Concentrations of perfluorinated compounds in whole blood in ng/g

Sample code: 5200	6066-01	6066-02	6066-03	6066-04	6066-05
Unit	ng/g blood	ng/g blood	ng/g blood	ng/g blood	ng/g blood
perfluorooctane sulphonate (PFOS)	0.2	<	0.2	0.1	0.1
perfluorooctanoic acid (PFOA)	int.	int.	int.	int.	1.4
perfluorooctane sulphonamide (PFOSA)	0.1	0.1	0.1	0.2	0.1

Sample code: 5200	6066-06	6066-07	6066-08	LOD
Unit	ng/g blood	ng/g blood	ng/g blood	ng/g blood
perfluorooctane sulphonate (PFOS)	0.2	0.2	<	< 0.1
perfluorooctanoic acid (PFOA)	1.1	2.2	2.3	< 0.2
perfluorooctane sulphonamide (PFOSA)	0.1	<	<	< 0.1

4.7 Metals

Metals are widely distributed in our environment and their accumulation can cause health damage. This is particularly true for lead, mercury and cadmium. Lead migrated in our food chain predominantly via lead tetraethyl contained in gasoline and tin-lead batteries, both that are more or less phased out by now.

Cadmium is popular due to its resistance to corrosion as has been used in colour additives. With mercury it is important to differentiate between organic and inorganic mercury compounds. While the latter cause acute toxic poisoning, the first cause motor and sensory disorders.

The results for the metals are reported in table 7 expressed in ng/g whole blood. Cadmium was found in 3 samples in concentrations up to 0.4 ng/g. Lead was found in all samples in concentrations ranging from 8.0 to 41 ng/g. Mercury was found in 5 of the 8 samples in concentrations up to 3.6 ng/g. The results for cadmium and lead are comparable to those reported in other studies while the results for mercury appear to be somewhat higher in this study.

Table 7. Concentrations of metals in whole blood in ng/g

Sample code: 5200	6066-01	6066-02	6066-03	6066-04	6066-05
Unit	ng/g blood	ng/g blood	ng/g blood	ng/g blood	ng/g blood
cadmium (Cd)	0.4	0.2	<	<	<
lead (Pb)	20	15	9.0	21	41
mercury (Hg)	1.4	<	<	1.5	3.6

Sample code: 5200	6066-06	6066-07	6066-08	LOD
Unit	ng/g blood	ng/g blood	ng/g blood	ng/g blood
cadmium (Cd)	<	0.4	<	<0.2
lead (Pb)	35	8.0	11	<1.5
mercury (Hg)	1.3	<	1.3	<0.8

5. QA/QC STATEMENT

TNO-B&O operates in compliance with the quality standard ISO 9001, certificate number 07246-2003-AQ-ROT-RvA. This study was performed in accordance with that ISO-9001 accreditation.