STICHTING TOT BEHOUD VAN TUINEN IN BUDEL-DORPLEIN

Aan: College van Gedeputeerde Staten van Noord-Brabant.

Geacht College,

Hierbij gelieve U aan te treffen een schrijven onzerzijds gericht aan de Inspecteur voor de Volksgezondheid en Milieu-hygiene voor de Provincie Noord-Brabant.

Alhoewel het schrijven voor zichzelf spreekt, verzoekt de Stichting U er op toe te zien, dat de Heer Inspecteur de nodige acties onderneemt en en hem daarbij ondersteunt.

Inmiddels verblijft de Stichting, ook in afwachting van activiteiten uwerzijds,

Hoogachtend,

voor de Stichting,
de secretaris,

A.K. van der Wal.

Secretariaat:
Parkdreef 113
6024 AG Budel-Dorplein.
04950-18417

p.s.
er is al een exemplaar ingeboekt

Budel-Dorplein 16-6-1992

OPEN BRIEF

Aan: Regionaal Inspecteur van de Volksgezondheid
voor de Milieuhygiëne Noord-Brabant,
dr.H.A.M.A. de Vries,

Betreft: Bodemsanering Budel-Dorplein, fase 1b.

Geachte Heer de Vries,

De Stichting zou gaarne het volgende onder uw aandacht willen brengen:

Naar aanleiding van het gehouden "vergelijkend bevolkings-onderzoek" door het R.I.V.M. rapport nr. 528303010 d.d. januari 1987 en het schrijven d.d.18 februari 1987, mede door U ondertekend, is een bodemsanering in Budel-Dorplein gefaseerd ingezet.

Zowel het vergelijkend bevolkingsonderzoek alsmede uw aanbeveling waren gericht op de effecten van cadmium op de volksgezondheid.
Dat heeft geresulteerd in een saneringswaarde van 2,5 mg.cd/kg dr.stof.
Vervolgens heeft de Provincie Noord-Brabant de mogelijkheid geboden aan de tuinbezitters, van meer dan 2,5 mg.cd/kg.dr.stof en minder dan 20.mg.cd/kg.dr stof in hun tuin, een overeenkomst aan te gaan tot vrijstelling van sanering - onder voorwaarden -.

Aangezien er geen protest of verweer tegen deze overeenkomst uwerzijds verschenen is, mag er van uit gegaan worden dat U met deze overeenkomst (en voorwaarden) accoord bent gegaan.

Voor alle duidelijkheid is te stellen dat het vorengenoemde vergelijkend bevolkingsonderzoek, de conclusies hierop en uw aanbevelingen dienaangaande, geleid hebben tot de sanering in Budel-Dorplein met de, nu afgesloten, fase 1a.

Het nadere bodemonderzoek in Budel-Dorplein van de percelen liggende in fase 1b., proj.nr: 5623-45868 nov.1991, geven opmerkelijke resultaten aan:

Slechts op één perceel van alle 85 onderzochte percelen is één cadmiumwaarde (te weten 25 mg.cd./kg dr.stof) gevonden boven de C-waarde van de toetsingstabel (20 mg.cd/kg.dr.stof).

In deze zin zouden alle verdere 84 percelen voor een overeen- komst van vrijwaring van sanering, onder voorwaarden, in aanmerking komen

Daarbij is te stellen, dat het ernstige gevaar voor de volksgezondheid, als bedoeld in de "Interimwet Bodemsanering" I.B.S., voor cadmium van de percelen onder fase 1b. niet aanwezig is.

De andere elementen aanwezig in de bodem van de percelen van fase 1b. te weten: zink, lood en arseen geven gemiddeld iets hogere waarden aan dan overeenkomstig in de percelen van de fase 1a.

Waar het echter aan ontbreekt is een gefundeerd breed vergelijkend bevolkingsonderzoek naar de effecten van deze stoffen, zink, lood en arseen, op de volksgezondheid in de zin van de titel I.B.S. : "ernstig gevaar voor de volkszondheid".

Rapporten in deze zin zijn:

- Het verslag van het Ministerie van Volksgezondheid en Milieuhygiëne d.d. april 1980 betreffende een onderzoek naar de lood-en cadmiumbelasting van 50 kinderen van de St.Andreasschool in Budel-Dorplein.

Uit de samenvatting, bladzijde 1, twee citaten:
1. "De loodgehalten in het bloed varieerden van 5 tot 21 microgram lood per 100 milliliter bloed.
Die resultaten liggen ruim beneden het Europese referentieniveau en ook nog ruim beneden de strengere grenzen die sinds kort voor kinderen wordt aangehouden".
2. "Noch ten aanzien van lood, noch ten aanzien van cadmium bestaat er reden tot zorg over de gezondheid van de inwoners van Budel".

Op bladzijde 13 van voornoemd verslag wordt deze conclusie, meer specifiek, betrokken op de onderzochte kinderen in Budel-Dorplein.

- Ten aanzien van de "humane toxicologische waarde" hierbij als bijlage een uitreksel van het proefschrift d.d. 24-9-'81 van H.W. Prins, T.H. Delft.

Hierbij worden de "de-toxificerende" eigenschappen aangetoond van het proteïne "metallothioneïne" ten aanzien van zink waardoor de meerdere aanwezigheid van (zware) metalen geen nadelige effecten hebben op de volksgezondheid.
Dit te stellen ten aanzien van de titel van de I.B.S., "ernstig gevaar voor de volksgezondheid".

De vrijwel permanente aanwezigheid van zink bij de andere voorkomende metalen, meer specifiek in Budel-Dorplein, is zelfs een gunstige voorwaarde.

Alvorens de Provincie Noord-Brabant in Budel-Dorplein een bodemsanering inzet uitgaande van het vergelijkend bevolkingsonderzoek op de effecten van cadmium op de volksgezondheid en uw aanbevelingen dienaangaande, gelijk fase 1a, is het niet alleen wenselijk, maar in zekere zin zelfs noodzakelijk, een vergelijkend bevolkingsonderzoek te starten daar het accent van de verontreiniging **niet meer** ligt op de meerdere aanwezigheid van cadmium, **maar op** zink, lood en arseen.

Het verslag van het Ministerie van Volksgezondheid en Milieu-hygiëne d.d. april 1980 is voldoende wetenschappelijk gefundeerd om generaal de conclusie te wettigen dat noch het cadmium noch de andere verontreinigingen in fase 1b. aanleiding zijn tot een volledige sanering van fase 1b. en zou volstaan kunnen worden met een generale ontheffing.

Alsmede de conclusies die getrokken kunnen worden uit de "de-toxificerende" werking van het aanwezige metallothioneïne ten aanzien van de anwezige zware metalen.

De Stichting gaat er van uit dat U, in kwaliteit én verant-woordelijkheid als Regionaal Inspecteur van de Volksgezondheid voor de Milieuhygiëne voor Noord-Brabant, gerekend af nu, de nodige stappen en maatregelen zult nemen.

Inmiddels verblijft de Stichting in afwachting van bericht en/of maatregelen uwerzijds,

hoogachtend, voor de Stichting,
namens de voorzitter,

J.M.A. Kooijman.

de secretaris,

A.K. van der Wal.

Secretariaat:
Parkdreef 113
6024 AG Budel-Dorplein
04950-18417

Bijlage: Uittreksel proefschrift H.W. Prins.

copie naar:

Minister, Mininisterie V.R.O.M.
G.S. Provincie Noord/Brabant
Gemeente Budel
Media

Bijlage brief d.d. 16-6-1992 Insp.Milhyg.Volksgez. Noord-Brabant.

Metallothionein Proefschrift H.W. Prins TH Delft 24/9-1981 pg 11

In 1957 metallothionein was discovered as a cadmium- and zinc-binding protein in the equine kidney (Margoshes & Vallee). Referring to the characteristic features of metal-binding and high cysteine concentration this protein was named metallothionein (Kägi & Vallee, 1960).

The terminology employed for different metallothioneins is confusing. In this thesis the recommendations for the nomenclature of metallothionein, as described by Kägi & Nordberg (1979), are used:

- the term metallothionein refers to all proteins which are characterized by:
  - molecular weight of 6,000 - 7,000 dalton (on gel filtration an apparent molecular weight of 10,000 dalton);
  - high metal content;
  - optical features of metal thiolates;
  - unique amino acid sequence;
- the term apometallothionein is used to specify the apoprotein (the polypeptide chain denuded of all metals);
- more specific terms such as "cadmium-metallothionein" and "copper-thionein" are used when metallothionein contains predominantly cadmium and copper, respectively;
- in contexts where specifications of the metal composition is not available or is of no special interest, the term "metallothionein" is used.

For unambigous identification it is recommended that the resemblance of a specific protein to equine renal metallothionein is documented by an analysis of its amino acid composition or of its amino acid sequence. The term "iso-metallothionein" is applied to those forms of metallothionein which occur in a single organism but are coded by different genes: they differ in amino acid composition.

Research was focussed on metallothionein when Piscator (1964) postulated that the synthesis of this protein was induced by cadmium and that it served as an intracellular cadmium-detoxifying protein.

Recently, a group of participants of the "First International Meeting on Metallothionein and other Low Molecular Weight Metal-binding Proteins" has reviewed the knowledge of these proteins (Kägi & Nordberg, 1979). Metallothionein is now thought to bind cadmium, zinc, copper, mercury, silver, bismuth and probably gold and lead depending on the metal to which the organism has been exposed. A number of biological functions has been proposed for metallothionein, viz., storage, detoxification and transport of metals, immune response and the metabolism of essential trace elements.

I.4.1 Biochemical features of metallothionein

I.4.1.1 Isolation of metallothionein

Metallothionein has been isolated from various tissues of a large variety of species ranging from microorganisms and crustacea to mice, rat and man (Kägi & Nordberg, 1979).

After homogenization and centrifugation, the standard isolation procedure (Webb, 1979) continues with the separation of the cytoplasmic proteins according to their molecular weight on Sephadex G-75. The protein fraction with a molecular weight of 10,000 dalton, which contains metallothionein, is further purified by ion exchange chromatography on DEAE Sephadex A-25, applying a gradient of Tris-buffer or of NaCl in Tris-buffer. Normally, cadmium- and zinc-metallothionein are separated in two isometallothioneins (Cain & Holt, 1979) and copper metallothionein in three different isometallothioneins (Bremner & Young, 1976). However, different ion exchange elution patterns have been described in literature (Suzuki & Yamamura, 1979; Webb, 1979). The differences in the elution patterns can originate from differences in the copper to zinc ratio of the isometallothioneins (Cain & Holt, 1979).

Recently, a new isolation procedure has been described using high pressure liquid chromatography (Suzuki, 1980; Suzuki & Yamamura, 1980a). In one step the metallothionein fraction of a crude supernatant is separated into the various isometallothioneins using a gel permeation column (TSK GEL SW 3000). The time for isolation is reduced from 2 days to less than 1 hour.

I.4.1.2 Amino acid composition and sequence of metallothionein

Metallothionein has a typical amino acid composition (Kojima & Kägi, 1978). It has a high concentration of cysteine (25-30%) and a relatively high concentration of lysine (10-15%) and serine (7-17.5%). Aromatic amino acids and histidine are absent. The differences in the amino acid composition of metallothionein described in literature are probably caused by differences in tissue from which metallothionein has been isolated, and in pureness of the preparation used for the determination of the amino acid composition (Webb, 1979).

The metallothionein polypeptide chain contains 61 amino acids (molecular weight 6,000 - 7,000 dalton) among which 20 cysteinyl residues, one methionine residue as the N-acetyl derivative at the N-terminal side and an alanine residue at the C-terminal side of the polypeptide chain. The cysteinyl residues occur in three arrangements, viz., cys-x-cys (7), cys-cys (3), and cys-x-x-cys (3); x represents an amino acid other than cysteine and the figure in parentheses represents the number of the arrangements. Basic amino acids and serine are predominantly located at the juxtaposition of the cysteinyl residues. Metallothionein does not contain free sulfhydryl or disulfide groups. The discrepancy in the molecular weight of metallothionein based on gel filtration experiments (10,000 dalton) and the molecular weight of 6,000 - 7,000 dalton calculated from the amino acid composition is caused by the non-globular shape of metallothionein, which results in a higher apparent molecular weight in gel filtration chromatography (Rydén & Deutsch, 1978).

Recently, Suzuki and Yamamura (1980b) described a dimer of cadmium-metallothionein. The dimers are formed by an intermolecular bond between two monomers and consist of the three combinations of the two isometallothioneins. Whether the dimers are isolation artifacts or are present under physiological conditions remains obscure.

I.4.1.3 Metal-binding in metallothionein

Zinc and cadmium are probably bound to metallothionein through mercaptide bonds with a cys-x-cys arrangement. The metals are chelated to a third cysteinyl residue as result of tertiary structure folding. The ratio of the cysteinyl residues to the sum of metal ions is 3, indicating that 6-7 metal-binding sites are available for these metals (Webb, 1979). A ratio of 2 found for copper suggests that there are 10 copper-binding sites.

From the fungus, *Neurospora crassa*, a copper-binding protein has been isolated with a molecular weight of 2,200 dalton (25 amino acids). The polypeptide chain is similar to the N-terminal region of the polypeptide chain of metallothionein. The ratio of the cysteinyl residues to copper is close to 1 for this protein (Lerch, 1980).

Metals differ in their affinity for metallothionein. The affinity decreases in the order: Hg > Ag > Cu > Cd > Zn. In vivo and in vitro, the metals bound to metallothionein are exchangeable for metals with a higher affinity. The apoprotein is obtained by acidification of zinc- or cadmium-metallothionein to pH of 4.5-3.5 and 3.5-2.5, respectively. Copper is removed at pH 1 by treat-

thionein, indicating that the polypeptide chains of these proteins are partially similar (Madapallimatam & Riordan, 1977). However, not all features of these proteins can be explained by the above mentioned mechanisms of artifact formation. Further research is required to elucidate the occurrence of the various low molecular weight copper-binding proteins.

I.4.2 Metabolism of metallothionein

I.4.2.1 Occurrence of metallothionein

Without prior induction with metals, minor amounts of (native) zinc-metallothionein are present in most tissues of an organism (Webb, 1979). After administration of metals the concentration of metallothionein remains constant due to the replacement of the native metal, zinc, by the administered metal. However, when the binding capacity of native metallothionein is exceeded the synthesis of metallothionein is induced (see section I.4.2.2; Webb, 1979).

Zelazowksi and Piotrowski (1977) measured the metallothionein levels in animal tissues. The metallothionein concentration varies within an organism (pig: liver 496 ± 50, muscles 12 ± 6 µg metallothionein/g tissue) but also between the various species (liver: pig 496 ± 50, rabbit 54 ± 22 µg metallothionein/g tissue). As function of age the metallothionein concentration of tissues varies as result of changes in the metabolism of trace elements. Copper accumulates in the liver in the perinatal period of life (Linder & Munro, 1973; Prins & Van den Hamer, 1978: paper II) and cadmium accumulates during lifetime in liver and kidney (Suzuki et al., 1979). Both changes in trace element metabolism are reflected in a higher metallothionein concentration (Piotrowski & Mogilnicka, 1976; Bell, 1979).

I.4.2.2 Induction of metallothionein

Initially, it was thought that metallothionein was synthesized in the liver and transported to the kidney and other tissues (Piscator, 1964). Since it was discovered that a large variety of isolated cells synthesize metallothionein after metal induction, it is generally accepted that almost every cell is able to synthesize metallothionein (Webb & Daniel, 1975; Kägi & Nordberg, 1979).

After exceeding an intracellular threshold in the metal concentration, the de novo synthesis of metallothionein is induced. The rate of synthesis of cadmium-metallothionein is within limits proportional to the administered cadmium dose and reaches a plateau at high metal concentrations (Sabbioni & Marafante, 1975). However, differences in tissue, sex, age and genetic predisposition are factors which influence the inducibility of the metallothionein synthesis (Kägi & Nordberg, 1979; Webb, 1979). Other factors, e.g., nutritional status (Bremner & Campbell, 1978), stress (Oh et al., 1978), glucocorticoids (Etzel et al., 1979) and alkylating agents (Kotsonis & Klaassen, 1979) primarily change the intracellular metal concentration which in turn induces the metallothionein synthesis.

Since metallothionein contains sulfhydryl groups, it has the potential to provide protection against alkylating agents (chemical-mediated toxicity). However, in comparison with glutathion metallothionein does not represent under normal conditions a detoxification pathway for alkylating agents (Cagen & Klaassen, 1980).

In rat after induction with cadmium, the synthesis of hepatic metallothionein starts after a lag-time of 2 hours. The rate of synthesis is maximal between 4 and 10 hours post injection and the metallothionein concentration reaches a maximum seven hours post induction. The induction of hepatic metallothionein synthesis is blocked by inhibitors of the DNA transcription, e.g., actinomycin D, and of the mRNA translation, e.g., cycloheximide. These results (Shaikh & Smith, 1977) suggest that the biosynthesis of metallothionein is regulated at the transcriptional (DNA) level. However, the induction is exclusively blocked when the inhibitor is administered prior to or simultaneous with the metal. Superinduction of zinc-metallothionein occurs when actinomycin D is administered while the zinc-metallothionein concentration is maximal post induction (Day et al., 1978).

In the kidney of the rat, the de novo synthesis of metallothionein starts one hour after the induction with metals. A maximum rate of synthesis occurs after 2-4 hours. Actinomycin D treatment does not inhibit the synthesis of metallothionein, indicating that the biosynthesis of renal metallothionein is regulated at the translational (mRNA) level (Shaikh & Smith, 1977).

I.4.2.3 Catabolism of metallothionein

Orally administered metallothionein is partly degraded in the intestinal lumen and is partly absorbed as the original metalloprotein in the intestinal mucosal cell and is also transported as such to the kidney cell (Cherian, 1979).

Parenterally administered metallothionein is predominantly transported to the kidney. The renal uptake of ^{35}S-labeled copper-metallothionein is essentially complete within 10-30 min and the concentration is constant for at least two hours. After two hours, 26.5% of the radioactivity is recovered from the kidneys and 30.8% from the urine; only 2.7% is taken up by the liver (Bremner et al., 1978a).

For the description of the catabolism of metallothionein, appears necessary to distinguish between the catabolism of the metal and that of the protein moiety of metallothionein. In reference to half-lives of the protein moiety, metallothioneins can be divided into two groups, viz., copper- and zinc-metallothioneins ($t_{½}$: 12-20 hours) and cadmium-metallothionein ($t_{½}$: 2-5 days) (Kägi & Nordberg, 1979).

In vitro, the protein moiety of zinc-metallothionein is twice as susceptible to degradation by hepatic lysosomal extracts than cadmium-metallothionein. Apometallothionein is degraded extremely rapidly (Feldman et al., 1978a). Since the polypeptide chains of zinc- and cadmium-metallothionein are similar (Vasak et al., 1980) this indicates that the metals bound to metallothionein influence, at least in vitro, the degradation rate of metallothionein.

In vivo, metal and protein moiety of copper- and zinc-metallothionein have equal half-lives, viz., 12-20 hours (Kägi & Nordberg, 1979). However, from the experiments of Terao & Owen (1973) with carrier free ^{67}Cu, one can calculate a half-life of less than 6 hours for ^{67}Cu bound in the protein fraction, with a molecular weight of 10,000 dalton, which is probably metallothionein. This could indicate that the amount of copper used in the experiments affects the half-life of metallothionein. This effect will be discussed further in section III.1.1.

The half-lives of cadmium and the protein moiety of cadmium-metallothionein are not equal (Feldman et al., 1978b). The protein moiety is degraded with a half-life of 2-5 days but cadmium is reincorporated in newly synthesized metallothionein and is excreted with a very long half-life.

All these results indicate that the inducing metal and the tissue in which metallothionein is synthesized influence the half-life of the metal and the protein moiety of metallothionein.

1.4.3 Metallothionein and trace element metabolism

Three types of metal can be distinguished in metallothionein. First, the inducing metal which is sequestered to avoid metal intoxication. Second, metals which are incorporated to stabilize the molecule; the half-life of copper-metallothionein is 12.3 h in zinc-deficient animals and 16.9 h in zinc-adequate animals (Bremner et al., 1978b). Third, the omnipresent metals which are bound to metallothionein after replacement of the native metal, zinc, due to differences in stability constants and in metal concentrations. Binding of non-inducing metals, i.e., the latter two types of metals, could result in antagonistic effects in trace element metabolism.

High dietary zinc uptake results in the rat in a decreased copper absorption from the intestinal tract (Osigo et al., 1974) and finally in symptoms of copper deficiency (Magee & Matrone, 1960). The high dietary zinc intake induces the synthesis of zinc-metallothionein in this organ. Zinc is replaced by copper which is apparent from the increased accumulation of ^{64}Cu in the intestinal metallothionein fraction as compared to control animals (Hall et al., 1979). Oral zinc therapy is used in Wilson patients in order to decrease the copper resorption from the alimentary tract (Hoogenraad et al., 1978).

Cadmium exposure results in an induction of renal metallothionein. In relation to the effect of cadmium induction on the copper metabolism, experimental animals could be divided into two groups (Suzuki, 1979). The first group, rat and guinea-pig, shows an increased renal copper-metallothionein concentration. In the second group, mouse, hamster and rabbit, the copper-metallothionein concentration is indifferent to the induction of renal metallothionein by cadmium. The origin of the difference between the two groups is unknown. But the results indicate that the presence or absence of effects of the induction of the metallothionein synthesis depends not only on the inducing metal but also on the animals used in the experiments.

half-life = halveringstijd = tijd nodig om de helft van de betreffende stof te doen ontleden