UNIVERSITY OF NOVA GORICA
GRADUATE SCHOOL

THE INTEGRATED EXPOSURE UPTAKE BIOKINETIC MODEL FOR THE LEAD BLOOD BURDEN PREDICTION IN CHILDREN AND ITS USEFULNESS IN STUDY AND REMEDIATION OF THE UPPER MEŽA VALLEY ENVIRONMENT

MASTER'S THESIS

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Nova Gorica, 2009

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Abbreviations

EPA: United States Environmental Protection Agency

WHO: World Health Organization

IARC: International Agency for Research on Cancer

CDC: Center for Disease Control and Prevention

ERICo: Environmental Research & Industrial Cooperation

OAQPS: Office of Air Quality Planning and Standards

ICRP: International Commission of Radiological Protection

BBU: Bleiberger Bergwerks Union

ALAD: δ-aminolevulinic acid dehydratase

ALA: δ-aminolevulinic acid

BpE: number of basophilic stippled cells

Rtc: reticulocyte count

Hct: hematocrit

ZnPP: zinc protoporphyrin

BLL: blood lead level

EDTA: ethylenediaminetetraacetic acid

PBPK models: Physiologically based pharmacokinetic models

IEUBK model: The Integrated Exposure Uptake Biokinetic model

UBK model: Uptake/Biokinetic model

GSD: geometric standard deviation

GM: geometric mean

CI: confidence interval

AAS: atomic absorption spectroscopy

ICP-MS: inductively coupled plasma mass spectroscopy

GFAAS: graphite furnace atomic absorption spectroscopy

CRM: certified reference material

IQ: intelligence quotient

1 INTRODUCTION

The Upper Meža Valley is situated in the north of Slovenia, near the Austrian border. It has a population of almost 7500. There are three important towns in the area: Črna na Koroškem, Mežica and Žerjav. This part of Slovenia has been an important industrial area with a 500-year mining tradition. The health studies conducted in the area in the past 50 years revealed large lead concentrations in the environment and large lead burden in the inhabitants.

1.1 Lead

Lead is a chemical element in the periodic table that has the symbol Pb (Latin: plumbum) and atomic number 82. A heavy, toxic, low melting metal (327.5°C), lead is bluish white when freshly cut but tarnishes to dull gray when exposed to air. Lead has a dull luster and is a dense, ductile, very soft metal that has poor electrical conductivity. Lead occurs naturally in the Earth's crust where it is usually found combined with two or more other elements to form lead compounds. The most important lead containing ores are galena (PbS), anglesite ($PbSO_4$), and cerussite ($PbCO_3$). Solubilities of lead compounds in water vary, lead sulfide and lead oxides being poorly soluble and the nitrate and chlorate salts are reasonably soluble in cold water. Lead is easily molded and shaped and also resistant to corrosion. When exposed to air or water, thin films of lead compounds (lead sulfate, lead oxides, and lead carbonates) are formed that protect the metal. It can be combined with other metals to form alloys.

Because of its characteristics lead was and still is used for many different purposes. It is used in building construction, lead-acid batteries, bullets and shot, and is part of solder, pewter, and fusible alloys. It is also found in pipes, weights, cable covers and sheets used to shield us from radiation. In the past lead compounds were used as a pigment in paints, dyes and ceramic glazes. Tetraethyl lead and tetramethyl lead were used as gasoline additives to increase octane rating. However in the last quarter of Twentieth Century because of its harm to the environment, use of lead in these products was suppressed or at least minimized. Most lead used by industry comes from mined ores ("primary") or from recycled scrap metal or batteries ("secondary").

Most lead today is “secondary” lead obtained from lead-acid batteries. It is reported that 97% of these batteries are recycled.[1,2,3]

1.1.1 Lead in the environment

Lead occurs naturally in the environment. However, most of the high levels found throughout the environment come from human activities. Environmental levels of lead have increased more than 1,000-fold over the past three centuries as a result of human activity. The greatest increase occurred between the years 1950 and 2000, and reflected increasing worldwide use of leaded gasoline. Lead can enter the environment through releases from mining lead and other metals, and from factories that make or use lead, lead alloys, or lead compounds. Lead is released into the air during burning coal, oil, or waste. Once lead gets into the atmosphere, it may travel long distances if the lead particles are very small. Lead is removed from the air by rain and by particles falling to land or into surface water. Once lead falls onto soil, it sticks strongly to soil particles and remains in the upper layer of soil. That is why past uses of lead such as lead in gasoline, house paint, and pesticides are so important in the amount of lead found in soil. Small amounts of lead may enter rivers, lakes, and streams when soil particles are moved by rainwater. Lead may remain stuck to soil particles or sediment in water for many years. Movement of lead from soil particles into groundwater is unlikely. It dependes: on rain pH value, on rain hardness, on the type of lead salt or compound and on the physical and chemical characteristics of the soil. Sources of lead in surface water or sediment include deposits of lead-containing dust from the atmosphere, waste water from industries that handle lead (primarily iron and steel industries and lead producers), urban runoff, and mining piles. Some lead compounds are changed into other forms of lead by sunlight, air, and water. However, elemental lead cannot be broken down. The levels of lead may build up in plants and animals from areas where air, water, or soil are contaminated with lead. Plants and animals may bioconcentrate lead, but lead is not biomagnified in the aquatic or terrestrial food chain.[2]

1.1.2 Applications of lead and its compounds

Lead has been used by humans for at least 7000 years, because it is widespread, easy to extract and easy to work with. In the early Bronze Age lead was used with antimony and arsenic. Lead was mentioned in the Book of Exodus (15:10). Alchemists thought that lead was the oldest metal and associated it with the planet Saturn. Lead pipes that bear the insignia of Roman emperors are still in service. Nowadays lead can be used in several areas for many different purposes. Here are some examples:

- Lead is a major constituent of the lead-acid battery used extensively in car batteries.
- It is used as a coloring element in ceramic glazes, notably in the colors red and yellow.
- Lead is used as projectiles for firearms and fishing sinkers.
- Lead is used in some candles to treat the wick to ensure a longer, more even burn.
- It is also used as shielding from radiation.
- Lead glass is composed of 12-28% lead oxide. It changes the optical characteristics of the glass and reduces the transmission of radiation.
- Lead is the traditional base metal of organ pipes, mixed with varying amounts of tin to control the tone of the pipe.
- It is used as electrodes in the process of electrolysis.
- It is used as sheathing material in high voltage power cables.
- Lead is used for the ballast keel of sailboats and also in scuba diving weight belts.
- Lead sheets are used as roofing material.
- Lead is added to brass to reduce machine tool wear.

Here are some applications from history:

- Tetraethyl lead was used in leaded fuels to reduce engine knocking.
- Lead was used as a pigment in lead paint for white as well as yellow and red colors. (lead chromate is still in use)
- It was used for plumbing already in Ancient Rome and to some extent (small diameter water pipes, cast-iron water pipes joints) until 1970s.
- It was also used as a component of toys.[1]

The largest use of lead within OECD countries in 1990 was for battery production, whereas there has been a large drop in the demand for lead-containing gasoline additives since 1970.[3]

In 2003, the U.S. consumption for lead was: 84.2%, storage batteries; 3.5%, ammunition, shot, and bullets; 2.6%, other oxides (paint, glass and ceramic products, other pigments and chemicals); 2.3%, casting metals (electrical machinery and equipment, motor vehicles and equipment, other transportation equipment, and nuclear radiation shielding); and 1.7%, sheet lead (building construction, storage tanks, process vessels, etc., and medical radiation shielding)**.**[1] Only 10 years ago still 13% of world lead consumption was for additives to fuel and other chemicals. That represented around 400.000 tons of lead annually.[4]

World reserves of lead are gradually declining and price in the market is rising. That rise is obvious since 2003. Although more and more lead is produced by recycling in US that still can’t meet market demands, especially in fast developing countries.[5]

1.1.3 Exposure to lead

One of the most important questions that should be answered is where one could be exposed to lead. Lead is commonly found in soil especially near roadways, older houses, old orchards, mining areas, industrial sites, near power plants, incinerators, landfills, and hazardous waste sites. People living near hazardous waste sites may be exposed to lead and chemicals that contain lead by breathing air, drinking water, eating foods, or swallowing dust or dirt that contain lead. People living in areas where there are old houses that have been painted with lead paint may be exposed to higher levels of lead in dust and soil. Similarly, people who live near busy highways may be exposed. Since lead compounds were formerly used as herbicides, insecticides, or rodenticides also people who live on old orchard land may be exposed. People may also be exposed to lead when they work in jobs where lead is used. Foods and vegetables may contain small amounts of lead. Leafy fresh vegetables may have lead-containing dust on them and should be washed well before consuming. Lead may also enter foods if they are put into improperly glazed pottery or ceramic dishes and from leaded-crystal glassware, but that is less common in developed world nowadays. Children may be exposed to lead by hand-to-mouth contact after exposure to lead-containing soil or dust. Children can ingest lead-laden dust through normal mouthing behaviors by simply placing their hand or an object in their mouth. This also happens when children handle food during eating.[6,7] In general, very little lead is found in drinking water. However, the amount of lead taken into your body through drinking water can be higher in communities with acidic water supplies. Acidic water makes it easier for the lead found in pipes, leaded solder, and brass faucets to be dissolved and to enter the water we drink. Plumbing that contains lead may be found in public drinking water systems that are older than 20 years. Breathing in, or swallowing airborne dust and dirt, is another way you can be exposed to lead. In 1984, burning leaded gasoline was the single largest source of lead emissions. Very little lead in the air comes from gasoline now because its use in gasoline for motor vehicles has been banned. Other sources of lead in the air include releases to the air from industries involved in iron and steel production, lead-acid-battery manufacturing, and non-ferrous (brass and bronze) foundries. Lead released into air may also come from burning of solid waste that contains lead, windblown dust, volcanoes, exhaust from workroom air, burning or weathering of lead-painted surfaces, fumes and exhaust from leaded gasoline, and cigarette smoke.

Skin contact with dust and dirt containing lead occurs every day. Recent data have shown that inexpensive cosmetic jewelry pieces sold to the general public may contain high levels of lead which may be transferred to the skin through routine handling.[8] However, not much lead can get into body through skin. In their homes people may be exposed to lead if they take some types of home remedy medicines that contain lead compounds. Some types of hair colorants, cosmetics, and dyes contain lead acetate.[9] People who are exposed at work (lead smelting and refining industries, battery manufacturing, lead compound manufacturing, brass/bronze foundries, construction and demolition workers etc…) are usually exposed by breathing in air that contains lead particles. Families of workers may be exposed to higher levels of lead when workers bring home lead dust on their work clothes and hair.[10]

Human exposure to lead above baseline levels is common. Some of the more important lead exposures have occurred as a result of living in environments with very dense traffic; in areas near smelters; consumption of produce from contaminated family gardens; renovation of homes containing lead-based materials; pica (an abnormal eating habit in children); occupational exposure; secondary occupational exposure; contact with interior lead paint dust etc… The highest and most prolonged lead exposures are found among workers in the lead smelting, refining, and manufacturing industries.

Lead released from natural sources, such as volcanoes, windblown dust, and erosion, are minor compared with anthropogenic sources. Industrial sources of lead can result from the mining and smelting of lead ores, as well as other ores in which lead is present. In these processes, lead may be released to land, water, and air. Electrical utilities emit lead in flue gas from the burning of fuels which contain lead. Because of the large quantities of fuel burned by these facilities, large amounts of lead can be released. Many of the anthropogenic sources of lead have been eliminated or phased out because of lead's persistence, bioaccumulative nature, and toxicity (lead in gasoline, lead based paint and pesticides). Because lead does not degrade, these former uses leave their legacy as higher concentrations of lead in the environment.[2]

1.1.4 Intake of lead into the body

Some of the lead that enters human body comes from breathing in dust or chemicals that contain lead. Most of the lead in ambient air is in the form of particles smaller than micron. Some 30–50% of these inhaled particles are retained in the respiratory system and virtually all of this retained lead is absorbed into the body. Particles from 1 to3 μm are also efficiently deposited in the lungs. Larger particles are deposited mainly in the upper respiratory tract with incomplete absorption. All lead particles that are cleared by the lung can be swallowed and result in further lead absorption from the gastrointestinal tract.[11] Lead may also be swallowed by eating food and drinking liquids that contain it. Most of the lead that enters body comes through swallowing, even though very little of the swallowed amount actually enters blood and other parts of body. The amount that gets into body from stomach depends on when last meal was eaten, on person's age and how well the lead particles dissolved in stomach juices. Experiments showed that, for adults who had just eaten, the amount of lead that got into the blood from the stomach was only about 6% of the total amount taken in. In adults who had not eaten for a day, about 60–80% of the lead from the stomach got into their blood.[12,13] In general, if adults and children swallow the same amount of lead, a bigger proportion of the amount swallowed will enter the blood in children, which absorb about 50% of ingested lead.[14]

Dust and soil that contain lead may also get on skin, but very small portion of the lead will pass through skin. More lead can pass through damaged skin (scratches, wounds). The additives in leaded gasoline could easily penetrate the skin are, but gasoline containing them is no longer sold to the general public. The most important part of lead on skin is that on the hands, because it can be accidentally swallowed when eating, drinking or smoking.[2]

1.1.5 Lead in the human body

Shortly after lead gets into body, it travels in the blood to the soft tissues and organs (liver, kidneys, lungs, brain, spleen, muscles, and heart). After several weeks, most of the lead moves into bones and teeth. In adults, over 90% of the total amount of lead in the body is contained in the bones, and around 73% of the lead in children's bodies is stored in bones.[15] Some of the lead can stay in bones for decades and some of it may reenter blood and organs under certain circumstances (during pregnancy and periods of breast feeding[16], after a bone is broken…). Human body does not change lead into any other form. Once it is taken in and distributed to organs, the lead that is not stored in bones leaves body in urine or feces. About 99% of the amount of lead taken into the body of an adult will leave in the waste within a couple of weeks, but only about 32% of the lead taken into the body of a child will leave in the waste.[15] Under conditions of continued exposure, not all of the lead that enters the body will be eliminated, and this may result in accumulation of lead in body tissues, especially bone.

1.1.6 Health effects of lead

Lead is a potent neurotoxin which accumulates in soft tissues and bone over time. Lead poisoning was recognized even by the ancients. Similarly, in the Twentieth Century certain dispersive uses of lead such as tetraethyl lead in gasoline, water pipe, solder in food cans, lead shot and sinkers, and in house paints, have been or are being phased out due to environmental and health concerns. In humans, lead can result in a wide range of effects. Effects at the subcellular level, as well as effects on the overall functioning of the body, have been noted and range from inhibition of enzymes to the production of marked morphological changes and death. Such changes occur over a broad range of doses, with children generally being more sensitive than the adults.[3]

Extreme lead exposure can cause a variety of neurological disorders such as lack of muscular coordination, convulsions and coma. Lower lead levels have been associated with changes in children's mental development and behavior.[17] These include: hyperactivity, deficits in fine motor function, hand-eye coordination, reaction time and lowered performance on intelligence tests. Chronic lead exposure in adults can result in: increased blood pressure, decreased fertility, cataracts, nerve disorders, muscle and joint pain and memory or concentration problems.[18]

Segments of the general population at highest risk of health effects from lead exposure are preschool-age children and pregnant women and their fetuses. Other segments of the general population at high risk include individuals living near sites where lead was produced or disposed and people which are exposed to lead in work place.[2]

The main target for lead toxicity is the nervous system. Long-term exposure of adults to lead at work has resulted in decreased performance in some tests that measure functions of the nervous system. Lead exposure may also cause weakness in fingers, wrists, or ankles. Lead exposure may also cause anemia.[19] At high levels of exposure, lead can severely damage the brain and kidneys and ultimately cause death. High levels of exposure to lead may cause miscarriage[20] in pregnant women or damage the organs responsible for sperm production in men. There is no conclusive proof that lead causes cancer in humans. Kidney tumors have developed in rats and mice that had been given large doses of some kind of lead compounds. The International Agency for Research on Cancer (IARC) has determined that inorganic lead is probably carcinogenic to humans. IARC determined that organic lead compounds are not classifiable as to their carcinogenicity in humans based on inadequate evidence from studies in humans and in animals.[21]

Studies of lead workers suggest that long-term exposure to lead may be associated with increased mortality due to cerebrovascular disease.[22] Population studies suggest that there is a significant association between bone-lead levels and elevated blood pressure.[23] Lead also affects kidney functions; glomerular filtration rate appears to be the function affected at the lowest blood lead levels. Decreased glomerular filtration rate has been observed in populations with elevated blood lead levels.[24]

Lead also alters the hematological system by inhibiting the activities of several enzymes involved in heme biosynthesis. Particularly sensitive to lead action is δ-aminolevulinic acid dehydratase (ALAD).[25] The anemia induced by lead is primarily the result of inhibition of heme synthesis and shortening of erythrocyte lifespan.

Children are more vulnerable to lead poisoning than adults. A child's exposure may be much different than an adult's exposure. Children eat more food, breathe more air and drink more fluids, per kilogram of body weight, and have a larger skin surface in proportion to their body volume. Also a diet of a child is different – it starts with placental nourishment, goes on with breast milk or formula and specific diet with of certain types of foods afterwards. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths, sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not use the judgment of adults to avoid hazards.

Children are exposed to lead all through their lives. They can be exposed to lead in the womb if their mothers have lead in their bodies and when they breast feed.[26] Babies can swallow lead if they eat foods and drink water that contains lead. Children can swallow and breathe lead in dirt, dust, or sand while they play on the floor or ground. These activities make it easier for children to be exposed to lead than adults. They are closer to the ground, where there is more dust, than adults. The dirt or dust on their hands and toys may have lead particles in it. Some children develop a habit of eating nonfood items which may contain large amounts of lead. Also, compared with adults, a bigger proportion of the amount of lead swallowed will enter the blood in children.[27]

Children are more sensitive to the health effects of lead than adults. Lead uptake by infant tissue is quicker, lead absorption rates are higher and clearance rates slower, meaning greater risk for children's health, especially in case of short–term or spike exposure. [28] No safe blood lead level in children has been determined. Lead affects children in different ways depending on how much lead a child swallows. A child who swallows large amounts of lead may develop anemia, kidney damage, colic, muscle weakness, and brain damage, which ultimately can kill the child. In some cases, the amount of lead in the child's body can be lowered by giving the child certain drugs that help eliminate lead from the body. If a child swallows smaller amounts of lead, much less severe but still important effects on blood, development, and behavior may occur. In this case, recovery is likely once the child is removed from the source of lead exposure, but there is no guarantee that the child will completely avoid all long-term consequences of lead exposure. At still lower levels of exposure, lead can affect a child's mental and physical growth. Fetuses exposed to lead in the womb, because their mothers had a lot of lead in their bodies, may be born prematurely and have lower weights at birth. Exposure in the womb, in infancy, or in early childhood also may slow mental development and cause lower intelligence later in childhood. One study also suggests that blood lead can also alter child's response to acute stress.[29] Children with high blood lead levels usually don`t have specific symptoms. However, health workers can find out whether a child may have been exposed to harmful levels of lead by taking a blood sample. They can also find out how much lead is in a child's bones by taking a special type of X-ray of the finger, knee, or elbow.

A study in children 8–10 years of age suggested that lead accelerates skeletal maturation, which might predispose to osteoporosis in later life. Lead also has been associated with increased occurrence of dental caries in children and periodontal bone loss. Lead also has been shown to decrease circulating levels of the active form of vitamin D, 1,25-dihydroxyvitamin D, in children with moderate to high blood lead levels (300–600 μg/l). Normal levels of vitamin D are important for maintaining calcium homeostasis. Two studies of children reported significant associations between blood lead levels and increases in serum IgE levels. IgE is involved in various allergic diseases such as asthma. These findings in children along with results from studies in rodents exposed in utero have led some to suggest that lead may be a risk factor for childhood asthma.[2]

Exposure to high amounts of lead (blood lead levels of 1000–1200 μg/l in adults or 800–1000 μg/l in children) produces encephalopathy.[30] Analyses of studies suggest

that an IQ decline of 1– 5 points is associated with moderate exposures to lead in early life.[31] Recent studies [32,33] have reported neurobehavioral deficits in children associated with blood lead levels under current level of concern (≥100 µg/l)[34] and an apparent lack of threshold down to even the lowest blood lead levels recorded in these studies.[35] These findings suggest reconsideration of the currently held notion that lead values in the range below 100 µg/l are acceptable from a public health perspective. In 2006 the Scientific Committee on Neurotoxicology and Psychophysiology and the Scientific Committee on the Toxicology of Metals of the International Commission on Occupational Health declared that current exposure standards for lead need urgently to be reduced. For children, the action level, which triggers community prevention efforts to reduce exposure sources, should be immediately reduced to a blood lead concentration of 50 µg/l in nations worldwide.
This level has been proposed as temporary level that may need to be revised further downward in future years as new evidence accumulates on toxicity at still lower blood lead levels.[36] In spite of these new findings the Centers for Disease Control and Prevention (CDC) did not decide to lower the current level of concern. The main reasons they point out are:

- No effective clinical interventions are known to lower the blood lead levels for children with levels less than 100 µg/l or to reduce the risk for adverse developmental effects.
- Children cannot be accurately classified as having blood lead levels above or below a value less than 100 µg/l because of the inaccuracy inherent in laboratory testing.
- Finally, there is no evidence of a threshold below which adverse effects are not experienced. Thus, any decision to establish a new level of concern would be arbitrary and provide uncertain benefits.[37]

There is some arguing going on that this decision by CDC is mostly politically influenced.[38] The CDC's claim about the inaccuracy in laboratory testing is mostly questioned one since there is enough proof showing otherwise. Studies indicate that a graphite furnace atomic absorption spectrometric (GFAAS) and inductively coupled plasma mass spectroscopy (ICP-MS) methods for the determination of lead in whole blood give very accurate results.[39,40] Nevertheless, the level of concern remains unchanged for the moment.

Studies of children also have shown associations between blood lead levels and growth.[41] Maybe the most important effects on health are neurodevelopmental effects. Lead can impair cognitive function in children and adults, but children are more vulnerable than adults. Beside specific characteristics which make them uptake more lead, perhaps more important is the fact that the developing nervous system is especially susceptible to lead toxicity. During brain development, lead interferes with the trimming and pruning of synapses, migration of neurons, and neuron/glia interactions. Alterations of any of these processes may result in failure to establish appropriate connections between structures and eventually in permanently altered functions.[2]

Evaluation of children exposed to lead with different subscales of IQ tests in conjunction with assessments of behavior on teacher's rating scales on young school-age children suggest that increased distractibility; impulsivity, short attention span, and inability to follow simple and complex sequences of directions are associated with increased lead body burden. Although the decrement of IQ points in children associated with lead exposure is generally small, lead neurotoxicity may have major implications for public health when exposure is considered in terms of large populations and its preventable nature. One study quantified the economic benefits from projected improvements in worker productivity resulting from the reduction in children's exposure to lead in the United States from 1976 to 1999. Based on data from National Health and Nutrition Examination Survey (data collected during 1976 through 1980, 1991 through 1994, and 1999) it was calculated that mean blood lead level declined 151 μg/l between 1976 and 1999. On the basis of published meta-analyses it was assumed that the change in cognitive ability resulting from declines in BLLs, should be between 0.185 and 0.323 IQ points for each 10 μg/l blood lead concentration. It was further estimated that each IQ point raises worker's productivity by 1.76–2.38%, and that the economic benefit for each year's cohort of 3.8 million 2-year-old children ranges from $110 to $319 billion.[42] One study estimated that mild mental retardation and cardiovascular outcomes resulting from exposure to lead amounts to almost 1% of the global burden of disease, with the highest burden in developing regions.[43] Adverse health effects of inorganic lead on children and adults are presented in following figure (Figure 1).

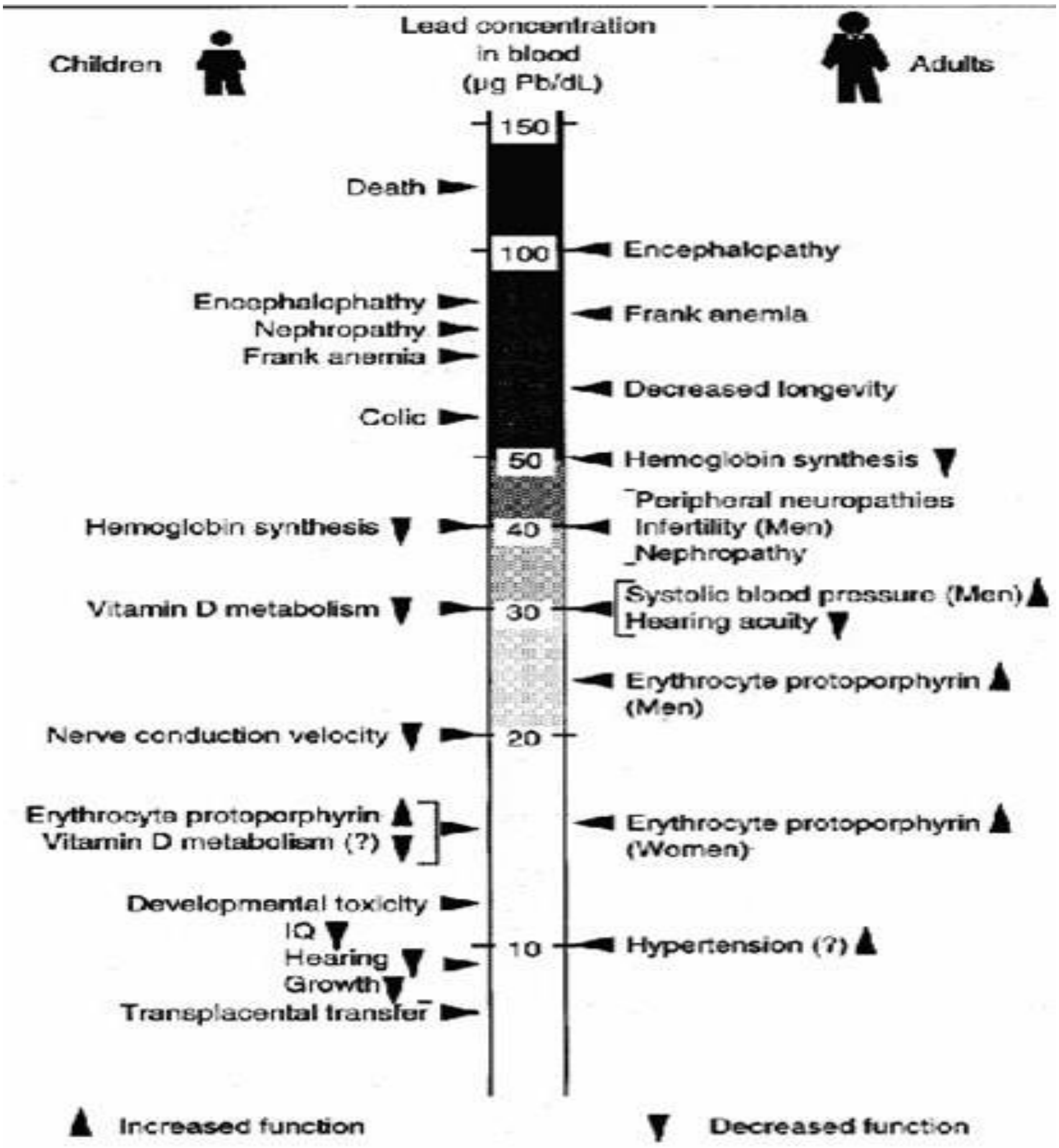


Figure 1: *Effects of inorganic lead on children and adults – lowest observable adverse health effects. (ATSDR, Toxicological Profile for Lead, 1989)*

1.2 Lead industry and its legacy in the Upper Meža Valley

The Koroška region is situated in the mountainous northern Slovenia along the Austrian border. It comprises heavily forested mountains and three narrow valleys along the Drava, Mislinja and Meža rivers. The Meža Valley is the most industrial of the three valleys. A steel mill Ravne na Koroškem operates in its lower part and a lead mine and smelter operated for many decades in its upper part. There are three towns in the Upper Meža Valley: Črna na Koroškem, Mežica and Žerjav. Mežica is the largest of the three with a population of about 3500, followed by Črna na Koroškem (2400) and Žerjav (480). A long-standing industrial history also leads to the polluted environment and successive health problems we are facing at present.

1.2.1 History of the lead mining in the area

The lead ore in the region was first mentioned in 1424 in the bookkeeping of the "Iron" Duke Ernest. He was the last to be appointed Duke of Carinthia according to the old usage and right. The ownership of small mines and smelteries was dispersed among licensed contractors by the mid 19th century. The production of lead saw a major growth between 1618 and 1648 during the Thirty Years War. The first still preserved written records about the lead ore exploitation date from 1665 when Hans Sigmund Ottenfels got a permit to open a lead mine near Črna na Koroškem. This year is also considered to be the actual beginning of lead mining in our Koroška region.[44] During Napoleon's Illyria, at the beginning of the 19th century, the lead mine in Mežica played a very important role – it was the only remaining lead mine in Austria since mines in Bleiberg and Rabelj (Cave del Predil) fell under the French. The mines situated beneath the Peca Mountain where exploited by Austria in order to obtain lead for ammunition.

The owners of the mine were numerous, but at the end of the 19th century, an Austrian company called Bleiberger Bergwerks Union, bought all smaller mines and this marked the beginning of the era of planned mining. The year 1893 can be considered the beginning since the building of new central smelteries started in Žerjav. The other smelteries in the area were gradually closed. Another important date from the standpoint of hygiene should be mentioned, i.e. 1899 when the first washroom was built in Žerjav. In year 1900, production of the Mežica mines topped production of the Bleiberg mine for the very first time.

Figure 2: *Group photo of miners at Helena shaft entrance, before The World War I (Archives of CPM – Gradbeni materiali d.o.o, Rudnik Mežica, http://www.podzemljepece.com/ 10.jun.2008)*

After the First World War, the Government of the Republic of Slovenia took over the mines and smelteries from the BBU. However, the BBU received protection from Great Britain and with the help of the firm Bewick Moreing Co. they founded the association of Central European Mines, Ltd. with its headquarters in London. During the occupation of Yugoslavia, the mines and smelteries continued to be the property of the BBU. However, with the founding of a new Yugoslavia after the Second World War in 1945, they were nationalized. The United Work Organization Mežica called “The Mine of Lead and Zinc Mežica” was established. In its peak, it used to employ over 2000 workers in various units, such as: the mine, the ore processing plant, the smelter, the battery factory, the factory of equipment, the saw mill etc... In 1988, the law demanding the closing down of the mine was elaborated. At the end of 1990, the United Work Organization was reorganized as a limited liability company and as such carried out the process of closure. The project on how to close down the mine was finished in 1994 and this is also the year when the production of lead and zinc ore were permanently stopped. It was then that the pumps for pumping water were stopped and the lower tunnels of the mine filled with water so that today the mine tunnels up to +417 m are under water. The superfluous water flows through the tunnel and comes to the surface in Prevalje. The mine closing programme was finished in 2000, with a monitoring program of closure works carrying on after that.[45]

1.2.2 The development of lead smelting in the Upper Meža Valley

Initially, lead production was rather primitive. The ore was stacked on wooden blocks, which were then ignited and the lead from the ore dripped to the bottom where it was collected and purified. In the furnaces which were still fired by wood 150 years ago, smelter workers roasted, mixed and pressed the lead ore without using any machinery. In this era, lead processing furnaces were very much scattered around the area.

After the First World War, mechanical equipment was gradually introduced into the Žerjav smelter, and the plant became completely mechanized by the middle of the 20th century. In 1962, a factory for storage batteries became an integral part of the plant. In 1989, the technology changed and the primary raw material, lead sulphide ore (galena), was replaced by a secondary raw material (old lead storage batteries). With the technological development, various filter systems were introduced in order to reduce lead emissions into the environment. The first filter system was introduced in 1923 and replaced by a new one in 1954. In 1964, a bag filter system was introduced. The largest improvement was achieved with a fiber glass filter system introduction in 1978 when the total amount of released dust dropped from 5,000 to 70 kg/day.[44] Better air cleaning systems after that further reduced dust emissions to less than 6 kg a day nowadays.[46]

Lead production capacity increased gradually from 5,556 tons per year in 1907 to 27,763 tons per year in 1977. For lack of own raw material and because of risk of environmental pollution, it dropped again to 16,000 tons per year. In spite of a significant reduction of lead emission due to the efficient filter system, the problem of environmental pollution with sulphur dioxide could only be resolved by discontinuing use of domestic lead sulphide ore and a switch to secondary raw material, mainly old storage batteries. This change in technology brought about a reduction in lead production capacity.[45]

Long years of mining and the expansion of the lead mine had a great impact on the area and life of the inhabitants in this part of Slovenia. This lead primarily to the rise in the number of miners and consequently the mine had a positive influence on the prosperity of the settlements Črna na Koroškem and Mežica. Nowadays, the polluted environment seems to be the most often mentioned consequence. About 19 million tons of the lead and zinc ores were dug out in total in a more than 800 km long tunnels in over three centuries.[47]

1.2.3 Lead related health studies in the past

Beside a 500-year mining tradition, the health studies have also been conducted for 50 years. Virtually all studies have shown elevated blood lead concentrations of the Upper Meža Valley inhabitants. In the studies done in the past, a gradual shift from the occupational exposure to general population and after that to more susceptible groups of population, can be observed.

At first, the studies focused on workers occupationally exposed to lead. First data on health risks and workers health protection date from the second half of the 19th century. The foundation of Brotherhood Found on 5 May 1863 is considered to be the start of organized social protection of miners and smelters. In 1912, health regulations for lead workers were published by the BBU. The regulations refer to three different degrees of lead poisoning with the following characteristics:

- I. Degree: conjunctivitis, anemia, lead pallor, lead line, gastritis
- II. Degree: lead colic, obstipation, trembling, paresis, sensoric disorders
- III. Degree: arthritis, circulation disorders, nefritis.

Dr. Simon Hohenwarter was appointed physician for miners and their families in 1903 in a small hospital at Črna na Koroškem. At that time, it was already understood that the ingestion of lead dust and inhalation of lead fumes caused lead poisoning. The diagnosis was based on a medical history and clinical picture, and treatment was palliative with emphasis on poisoning prevention. Periodical examinations were performed on smelter and refinery workers each month and in other workers at least every three months. Special attention was paid to personal hygiene. In 1899, washbasins, showers and a separate laundry-room were built in the Žerjav smelter. It was obligatory for workers to change clothes before and after work and to thoroughly wash the body after work. It was forbidden to smoke and chew tobacco in the working premises. Before meals, it was mandatory to wash the hands and to rinse the mouth. At work, the use of respirators was the rule particularly for those exposed to lead. The workers were regularly circulated in different work places.[45]

In 1927, when Dr. Marija and Dr. Adolf Ramšak started to work in the Črna hospital 48 patients suffered from heavy lead poisoning symptoms, some even had paralysis and encephalopathy. Five years later, monthly check-ups for workers in the smelter were introduced and the number of lead poisonings fell to 6-10 per year. In 1941, the hospital at Črna na Koroškem was extended. After that year, a rise in lead production was coupled with an increased number of poisonings. The diagnosis was based on a medical history, subjective complaints and characteristic clinical signs, such as lead line, lead pallor, trembling, weakness of the extensors and particularly the number of basophilic stippled cells (BpE). From 1958 onwards, intravenous injections of the chelating agent $CaNa_2EDTA$ have been used for treatment of lead poisoning. In 1956, the hospital at Črna na Koroškem was extended again. A list of contraindications for employing new workers in the smelter was prepared by Dr. Bogdan Dolenc. These included: women, older than 45 or younger then 21 if not previously exposed to lead, cardiovascular diseases, alcoholism, disorders in breathing through nose etc…. He also established the basis for clinical and laboratory examinations and he set up criteria according to which a worker could continue work, had to change his job for a less exposed one, or had to receive medical treatment (Degrees I to III). He delimited chelating therapy in three phases according to blood lead level: starting phase (1830 μg/l), critical phase (4220 μg/l) and end phase (600 μg/l). Dr. Dolenc also examined seven children in his practice. They had been poisoned by lead through contaminated air and water and treated them with chelating agent.[48] In that period, the first scientific conclusions were unveiled testifying that all Žerjav inhabitants absorb more lead and consequently children were forbidden to play near the smelter.

In 1959, an outpatients occupational health department was completed in the Žerjav smelter. Four years later the work of the department was taken over by the Health Centre Ravne na Koroškem at Mežica. At the same time, the hospital at Črna na Koroškem lost its last permanent physician, thus it joined the General Hospital in the town of Slovenj Gradec. From that time, physicians in the hospital at Črna na Koroškem alternated each month. In spite of the organized service, the hospital was closed down in 1967 due to economic reasons. Routine check-ups of workers in the outpatient department consisted of a clinical examination, hematological and biochemical tests. A major important criterion for poisoning was the BpE number.

The workers who in addition to a high BpE number also had subjective complaints and positive clinical signs of lead poisoning were treated with an intravenous injection of $CaNa_2EDTA$ for 3-4 days. In 1970, new criteria for lead poisoning severity (Degrees: I (normal), I-II (still allowed), II (excessive), and III (critical)) were worked out by Dr. Čegovnik based on coproporphyrin and δ-aminolevulinic acid (ALA) levels in urine, medical examination results and clinical signs of overexposure to lead or lead poisoning. Both, ALA and coproporphyrin, rise, when body is lead intoxicated. ALA increases at blood lead levels over 400 μg/l and coproporphyrin rises at even higher blood lead levels.[45] In 1984, the outpatients department at Mežica was taken over by Dr. Bojan Jezernik. He accepted the previous criteria for diagnosis and medical treatment, but replaced an intravenous injection with an infusion of chelating agent in treatment. It is interesting that some occupational health criteria were far below from those in other European countries. For example, criteria for temporary removal of worker from work place for δ-aminolevulinic acid in urine was 230 μmol/l, what was twice the amount used in Germany (115 μmol/l). Stricter criteria were under consideration, but it was believed that would result in a major reduction of workers thereby leading to decreased production. Dr. Jezernik realized that personal hygiene and the use of respirators had an important role in lead absorption.

Health surveillance of lead exposed workers in the Mežica Lead Mines and Smelter has been directly connected with the development of the health service and also with the knowledge of lead toxicodynamics and toxicokinetics. The oldest available data refer to the period 1894-1903. These document list colic together with other diseases that could be related to stomach cramps. The data showed that almost all cases included smelter workers rather than miners. From 1951 to 1962, the frequency of lead colic gradually decreased. The most severe poisoning occurred in smelter workers. In the 1962-1971 period, lead colic was registered in only one smelter worker. According to clinical and laboratory findings, more workers were classified in degree II (temporary removal to a less exposed job) than in degree III (treatment with a chelating agent). The 1972-1989 follow-up also included workers from the factory of lead storage batteries, none of whom showed signs of colic. According to criteria II and III classification, it was established that lead smelter workers were more exposed to lead than those from the storage battery factory.[45]

Several studies of lead absorption were also conducted in that period. The first was performed in 1952 among a total of 343 workers (222 smelter workers and 121 miners). Analysis of erythrocytes, hemoglobin, BpE, reticulocytes (Rtc), coproporphyrin and blood lead demonstrated higher lead absorption in lead smelter workers than in miners. The blood lead levels in that study were 1330 μg/l in smelter workers, 1000 μg/l in miners and 660 μg/l in the control group. This was in agreement with the lead content in the working atmosphere, which was also very high (0.2 – 10.2 mg/m^3).[49] In the 1971, 51 smelter workers were examined for blood lead, activity of ALAD, Hb with hematocrit (Hct), Rtc, BpE, ALA and coproporphyrin. All workers showed increased lead absorption. The blood lead levels were from 336 μg/l to 898 μg/l.[50] In the 1975 study, a total of 115 smelter workers were examined for blood lead, lead in urine, ALA and coproporphyrin. Normal values were exceeded in the majority of workers. In addition, some workers exhibited functional and organic capillary changes as well as changes in the eye background.[51] Next study was carried out in 1982 and it included 114 workers with different lead exposure. There were five groups according to different work place: production workers in metallurgic section, maintenance workers in metallurgic section, workers in battery industry, technical personnel and auxiliary personnel. Blood lead, ALAD, zinc protoporphyrin (ZnPP) and hemoglobin were analyzed in each subject. All workers and two thirds of technical and auxiliary personal showed increased lead absorption. A highly significant correlation was found not only for blood lead-ALAD and blood lead-ZnPP, but also for blood lead-hemoglobin indicating dangerously excessive lead exposure. Average blood lead levels for different groups were between 449 μg/l and 790 μg/l with individual values between 235 μg/l and 1330 μg/l.[52] In 1987, 79 workers with different lead exposure were examined for blood ALAD, erythrocyte protoporphyrin, urine ALA, and coproporhyrin. This time, three groups were formed: smelter workers, lead processing workers, and supervision and technical personnel. The study showed a rather high prevalence of increased lead absorption which was higher in both groups of workers.[53]

Health studies in the inhabitants of the Meža Valley also have already over 50 years of history. The first study of the effect of environmental metal pollution on the health of the Upper Meža Valley inhabitants began in 1952 and there are some studies still running. Most investigations were related to lead effects, a very small part referred to cadmium.

The first investigation in 1952 on 41 inhabitants of Žerjav, found the average blood lead concentration of 700 μg/l.[49] In 1968, the study included 912 subjects (men, women and children) without occupational exposure to lead. 61.3% of them had urinary ALA values higher than 10 mg/l, which was defined as a level of concern. In a sub sample of those with increased ALA values, the number of red blood cells and hemoglobin concentrations were within a normal range. These parameters were slightly decreased in preschool children. Rtc count was increased in 55.0 % of preschool children, 38.0 % of school children, 18.1 % of men and 34.2 % of women. The ALAD activity was decreased (< 70 units/cm^3) in 90.0 % of the exposed subjects compared with 6.6 % in controls (Mislinja). Coproporhyrin concentrations measured in 255 subjects were increased only in 18, while only six children had increased spontaneous excretion of lead in urine. A study including 300 inhabitants of the Meža Valley carried out between 1968 and 1973 showed that measured ALA values were in good accordance with the data for chimney filter system efficiency.[54,55]

Analyses of blood lead concentrations and some other markers of body lead burden carried out in 1972, and 1974-1976 on all members of representative groups of families demonstrated that in families where fathers did not have additional occupational lead exposure, the sequence of lead absorption was starting with highest: fathers followed by school children and preschool children followed by mothers.[56] The children whose fathers were occupationally exposed to lead had higher lead absorption than those with fathers whose exposure was only environmental.[57] In comparison with control groups, the inhabitants of the Meža Valley had increased lead absorption. The median blood lead concentration exceeded 200 μg/l for each group. The median blood lead concentration of preschool children was over 400 μg/l. After the introduction of efficient bag filters in 1978, which reduced the air lead concentration, a follow-up study of lead absorption intensity was performed on mothers, school children and preschool children. A trend towards normalization of blood lead concentrations was evident in each studied group.[58,59] A follow-up study also showed that children had higher lead absorption than their mothers in all groups. The children born after bag filter installation (1978) had similar lead absorption to those born before, indicating that lower air lead values were not followed by a similar trend of lead decrease in other environmental media (particularly soil and household dust). The inhabitants living within a distance of up to 1000 m from the smelter had the highest lead absorption and therefore it was suggested that this area should be transformed to an industrial one.

In 1982, a study including 35 pregnant women was carried out. The most important finding was that blood lead increased during pregnancy. This was explained by lead mobilization from bones, accumulated during greater lead exposure in the past. The increasing blood lead trend was also present during lactation, which also means that children were exposed during breastfeeding.[60]

An estimation of lead body burden by the use of EDTA lead provocation test was conducted in 1970, first on 109 inhabitants and later on additional 101 inhabitants (adults and children) of the Meža Valley. Lead in urine was determined before and after EDTA administration. The results showed that only 8.5 % of the subjects had a spontaneous excretion of lead higher than 100 μg/l, which was considered to be a normal level. After EDTA administration, excretion of lead was above 500 μg/l (a predicted value in non-occupationally exposed people) in 81.5 % of subjects in the first group, and in 78.4 % of subjects in the second group, ranging from 500 to 4200 μg/l.[45]

An assessment of lead absorption intensity by analysis of lead concentration in the hair was performed. Three groups have been formed - inhabitants of the Meža Valley, lead smelter workers and control group coming from an uncontaminated area (Mislinja). Average lead concentrations in the hair were the highest in smelter workers 71.90 μg/g, followed by inhabitants of the Meža Valley (33.13) and controls (3.76), showing a relatively good correlation with the level of lead exposure.[61]

A chromosomal investigation in inhabitants of the Meža Valley showed some changes in lymphocytes of peripheral blood. Defects were not numerous or severe, but they were not found in a control group from the uncontaminated area.[60]

The physiological effect of lead in children was studied in 1986. In a group of 40 children aged 5.5-8 years from the lead smelter area, and in nine children from the control area, an intelligence test, constisting of 5 subtests for evaluation of verbal intelligence and 5 subtests for evaluation of non verbal intelligence, was applied. Attention was determined through reaction time. A visual-motoric integration test was also performed. The results obtained were compared to the exposure data based on biological monitoring of the characteristic parameters (blood lead, lead in milk-teeth…). Some statistically significant correlations were determined, among them a negative correlation between verbal intelligence coefficient and blood lead (also teeth lead) concentration. Although the sample was very small, the results indicated a possible correlation between lead absorption and some physiological functions in the examined children, but investigations should be carried out on much larger sample for consistent results.

The incidence of ischemic heart disease, hypertensive disease, cerebrovascular disease, other diseases of the circulatory system and nephritis was studied in inhabitants of the Meža Valley and in a control area over a period of eight years (1966-1973). The data was taken from hospital treatment records and causes of death. Ninety married couples with the husband working in the lead smelter and 73 couples with the husband not employed in the smelter were medically examined. A group of 88 married couples from a control area was also examined. Although the results gained by different methodological approaches were not entirely consistent, comparative analysis indicated that ischemic heart disease, cerebrovascular disease and nephritis occurred more often in the population from the smelting area.

The abortion rate was studied separately. The rate of spontaneous abortions was higher in the smelter area than in the control area. Hospital data also indicated that the complications in pregnancy, delivery and puerperium were more frequent in that area. An additional retrospective analysis (1961-1980) also showed a higher rate of spontaneous abortions in the smelter area. Starting from 1978 when efficient bag filters were installed, the difference in the rate of spontaneous abortions between the two areas disappeared. Comparison of the rates of twin births for the two areas did not show any difference. A more detailed analysis, however, demonstrated a lower twin birth rate in the vicinity of the lead smelter than in the rest of the community. As the rate of twin births is inversely proportional to the rate of spontaneous abortions, this finding supports the assumption of the involvement of lead exposure in the effect on reproduction.[45]

With a rising interest in the environment, studies of environmental pollution also gain in importance. An investigation of environmental pollution of the Meza Valley by lead, zinc and cadmium caused by emissions from the lead smelter started in 1967 and has to some extent still continued. The data obtained from 1967 to 1978 during high emission rates pertaining to considerable environmental pollution and those from 1979 onwards, after an efficient bag filter system was installed to control the emission from the lead smelter, showed a marked reduction in pollution. The concentration of lead in the air was drastically reduced. In 1968, when measurement was started concentrations in some places were over 80 μg/m^3.[62] After 1978, when a new filter system was constructed, air lead concentration fell under 3 μg/m^3. It is also interesting that before filters were installed, the highest air lead concentrations were measured in Rudarjevo about 2 km southwest of the smelter stack. After 1978, highest concentrations were measured in Žerjav, also size distribution of lead particles has been changed towards larger particles indicating a higher contribution of dispersed dust. Both facts can lead to the conclusion that somewhat higher concentrations around the smelter (≤500m) come from low sources. Due to the climate characteristics of the area, lead concentrations in the air showed a marked seasonal pattern with maximum concentrations in winter (fewer precipitations and frequent inversions). Lead concentrations measurements in depositions also showed reduction in concentrations, but to a lesser extent. They varied from a year to year and the annual means at most sites were still above the then valid German limit value for urban and industrial areas (250 μg/m^2/day). Less reduced metal concentrations in deposited particles compared to airborne particles suggest the contribution of redispersed soil and road dust. Measurements of lead, zinc and cadmium content in soil support this assumption.[63,64]

Measurement of lead, zinc and cadmium concentrations in soil began in 1968 and was carried out intermittently until 1971. The samples were collected and total and soluble lead (as a measure of lead available for vegetation) was determined. The total lead content of the soil ranged from 1000 mg/kg in the remote parts of the Meža Valley to 25000 mg/kg in the close vicinity of the smelter compared to <20 mg/kg in the control area. The soluble proportion of lead was 3.5-66.0%.[65] In period from 1981 to 1988, the samples of soil were collected from three layers of 10 cm each (total depth 30 cm beneath the surface layer) every spring and autumn at 12 locations. The lead concentrations varied over the year, but without any particular trend, while cadmium concentrations showed a slight increasing trend. Both lead and cadmium in the soil were above the then valid German limit values (300 μg Pb/g and 1-2 μg Cd/g) at most locations and several times higher than at the control point (Ribnica na Pohorju). Particularly high levels were found close to the smelter and at the location which previously had maximum ground level concentrations before lowering of air pollution in 1978. Analysis results of soil samples obtained at the same location before 1971 and after 1981 showed that the concentration levels did not change much after the introduction of a new filter system in 1978.[63,64] If we take into account aspect results of the last study carried out by ERICo in 2002, we can only confirm that conclusion. In 1968, lead concentrations in soil were between 191 and 1580 mg/kg, they were between 163 and 2236 mg/kg in the period 1981-1988, and between 511 and 4470 mg/kg in 2002.[45,46]

Household dust was sampled and analyzed for lead, zinc and cadmium in 1975/76 and again in 1980-1990 in 10-15 homes. The samples were collected each year in late spring (May - June). The lead and zinc concentrations in household dust collected after 1980 were lower than in 1975/76, but cadmium concentrations after the decrease in 1980-1984 started to increase as they did in the soil. The highest concentrations were found close to the smelter. In 1975, average lead concentrations in house dust were almost 5000 mg/kg; from 1980 to 1990 they varied from around 1700 to around 3000 mg/kg.[45] In ERICos study from 2002, average concentration was 1134 mg/kg (524 – 2227 mg/kg).[46]

An analysis of metal content in surface waters carried out before waste water treatment was introduced, showed a drastic increase in lead content in the Meža after release of effluents from the smelter (from 0.007 up to 685 mg/l) with a gradual decrease up to the influx of the Meža into the Drava. Around 300 m from the place of release, lead content was 226 mg/l and around 10 km from it, the concentration dropped to 92 mg/l. Lead content in potable water never surpassed the current limit value of 10 μg/l and it was even lower than in the control area. Lead in snow was measured before abatement of pollution and it amounted to 0.02-274.9 mg/l in the polluted area compared to 0.01-0.07 mg/l in the control area.

Lead and cadmium were also measured in foodstuffs. Although the levels decreased after 1978 they were still higher than in the control area and higher in foodstuffs grown or bred in the polluted area than in those purchased in shops. Duplicate samples of diet were collected before and after 1978 in rural and urbanized households. The total dietary lead intake decreased after the introduction of control measures, but it was still higher than in the control area, especially in households close to the smelter. In order to assess the total intake of lead and cadmium, in addition to intake with food and beverages, the inhaled portion was also taken into account. For this reason, lead and cadmium were measured in the ambient air outdoors and indoors in 15 homes, the residents of which provided duplicate diet samples, in kindergartens, at school, in canteen and in an office over a period of seven days in winter and again in summer. The calculated total daily intake of lead ranged from 14 to 611 μg and of cadmium from 1.7 to 17 μg. The highest intake was again attributed to people living in the close vicinity of the smelter. It was recommended that no homes, gardens or grazing be permitted within 1000 m from the smelter.[45]

The last comprehensive study of lead and other heavy metals burden in the Upper Meža Valley addressing different areas from the environment to food, animals and people is The Comparative Study of the Environment Pollution in the Upper Meža Valley between 1989 and 2001. The study was carried out by ERICo Institute in the period 2001 – 2002. The study results showed that the concentrations of heavy metals and especially lead are still high and that environment remediation should be carried out.

1.3 The IEUBK model

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes. They are used in risk assessments, to predict the concentration of a certain toxic chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species.

The Integrated Exposure Uptake Biokinetic (IEUBK) Model for Lead in Children is one of the most recognized PBPK models for lead. IEUBK was designed by US EPA. It can be used for prediction of the elevated blood lead levels in children under the age of seven who are exposed to environmental lead from many sources. The IEUBK model is a PC compatible software package, allowing the user to estimate a plausible distribution of blood lead concentrations centered on the geometric mean blood lead concentration predicted by the model from available information about children's exposure to lead. The estimation can be done for a hypothetical child or population of children. The model also calculates the probability that children's blood lead concentrations will exceed the user selected level of concern (default ≥100 μg/l). The user can then explore an array of possible changes in the exposure media that would reduce the probability that blood lead concentrations would be above this level of concern.

There are many available data on human blood lead burden and environmental lead pollution for the Upper Meža valley. The data clearly show that the area is still polluted and that the inhabitant’s health can be threatened. Environment remediation is needed. By using the data with the IEUBK model, we expect that the model will be helpful in identifying the most important exposure factors on which remediation activities should focus. The model could also substitute blood sampling in young children when wanting to estimate children’s blood lead burden.

2 AIM, GOALS AND HYPOTHESIS

2.1 Aim

The aim of the combined activities, part of which is also our research, is to improve health of the Upper Meža Valley inhabitants. The main focus is on lowering the lead blood burden of children under the level of concern. To achieve it, we must reduce the human exposure and lead intake. It was our belief that the IEUBK model could be helpful in identifying the most important exposure factors and further in making decisions when choosing strategies for lowering lead exposure and intake.

2.2 Goals

With the aim of finding a usefull tool for estimating environmental health of the Upper Meža Valley inhabitants, we decided to test the IEUBK model with the lead pollution data for the Upper Meža Valley. Our goal was to evaluate function of the IEUBK model with data for our region. We wanted to know if the human lead burden data reflects environmental lead contamination. We were confident that the calculations with the IEUBK model would point out the most important exposure factors to lead in the Upper Meža Valley.

2.3 Hypothesis

Our hypothesis was: “The IEUBK model will act well, when using the environmental lead pollution data for the Upper Meža Valley, already for available input data. The predicted values for geometric mean blood lead and proportion of children with elevated blood lead levels will be in satisfying agreement with observed values.”

3 MATERIAL AND METHODS

The study used data from the Comparative study of the Environment Pollution in the Upper Meža Valley between 1989 and 2001 carried out by ERIC-o Institute in the period 2001 – 2002 and results from the project “Living with lead” conducted by the Regional Institute of Public Health Ravne na Koroškem. The first one provides the environmental lead burden data for calculations with the IEUBK model. The lead burden in children data from the project “Living with lead” was merged with the blood burden data from the ERICOs study. The lead in drinking water data, acquired from the Regional Institute of Public Health Ravne na Koroškem archives, were also used.

3.1 The IEUBK model

The Integrated Exposure Uptake Biokinetic (IEUBK) Model for Lead in Children is a PC compatible software package, which allows the user to estimate, a plausible distribution of blood lead concentrations centered on the geometric mean blood lead concentration predicted by the model from available information about children's exposure to lead. Estimation can be done for a hypothetical child or population of children. The model also calculates the probability that children's blood lead concentrations will exceed the user selected level of concern (default ≥100 μg/l). The user can then explore an array of possible changes in exposure media that would reduce the probability that blood lead concentrations would be above this level of concern.

The IEUBK model simulates lead uptake, distribution within the body, and elimination of lead from the body. The model has four major submodels:

- Exposure model calculates average daily intake of lead (μg/day) for each inputted environmental lead concentration (lead in air, diet, dust, soil, and water) and media specific consumption rate.
- Uptake model calculates the amount of lead which is absorbed into child’s bloodstream from Pb intake into the lungs and digestive tract. The uptake portion of the model takes into consideration two mechanisms of absorption of lead in the digestive tract: saturable and nonsaturable. It converts environmental media-specific lead intake rates calculated from the exposure

model into a media-specific time-averaged uptake rate (µg/day) of lead to the central compartment (blood plasma).

- Biokinetic model, which simulates the transfer of absorbed lead between blood and other body tissues, elimination of lead from the body (via urine, feces, skin, hair, and nails), and predicts an average blood lead concentration for the exposure time period of interest.
- Blood lead probability model, which applies a log-normal distribution (with parameters geometric mean and geometric standard deviation) to predict probabilities for the occurrence of a specified given blood lead concentration in a population of similarly exposed children. The module also calculates the probability or the risk that the level of concern (≥100 µg/l) will be exceeded.

Shown conceptually on Figure 3 inhaled or ingested lead is absorbed through the lungs or gut into the blood stream where it is transferred to body tissues, including bone tissues. After a period of time, this lead returns to the blood stream where it is transferred to other tissues or eliminated with urine. Lead may also be eliminated from the body with sweat, hair or sloughed epidermal tissue, or it may be transferred through the liver and bile duct back to the gut where it passes out of the body with feces. The oval shapes show environmental media and the pathways of uptake. The large rectangle is the blood plasma compartment central to the distribution of lead in the body. Each lower rectangle shows a compartment in the child's body where lead may be retained. The excretion of lead from the body is shown by the circle.[66,67,68]

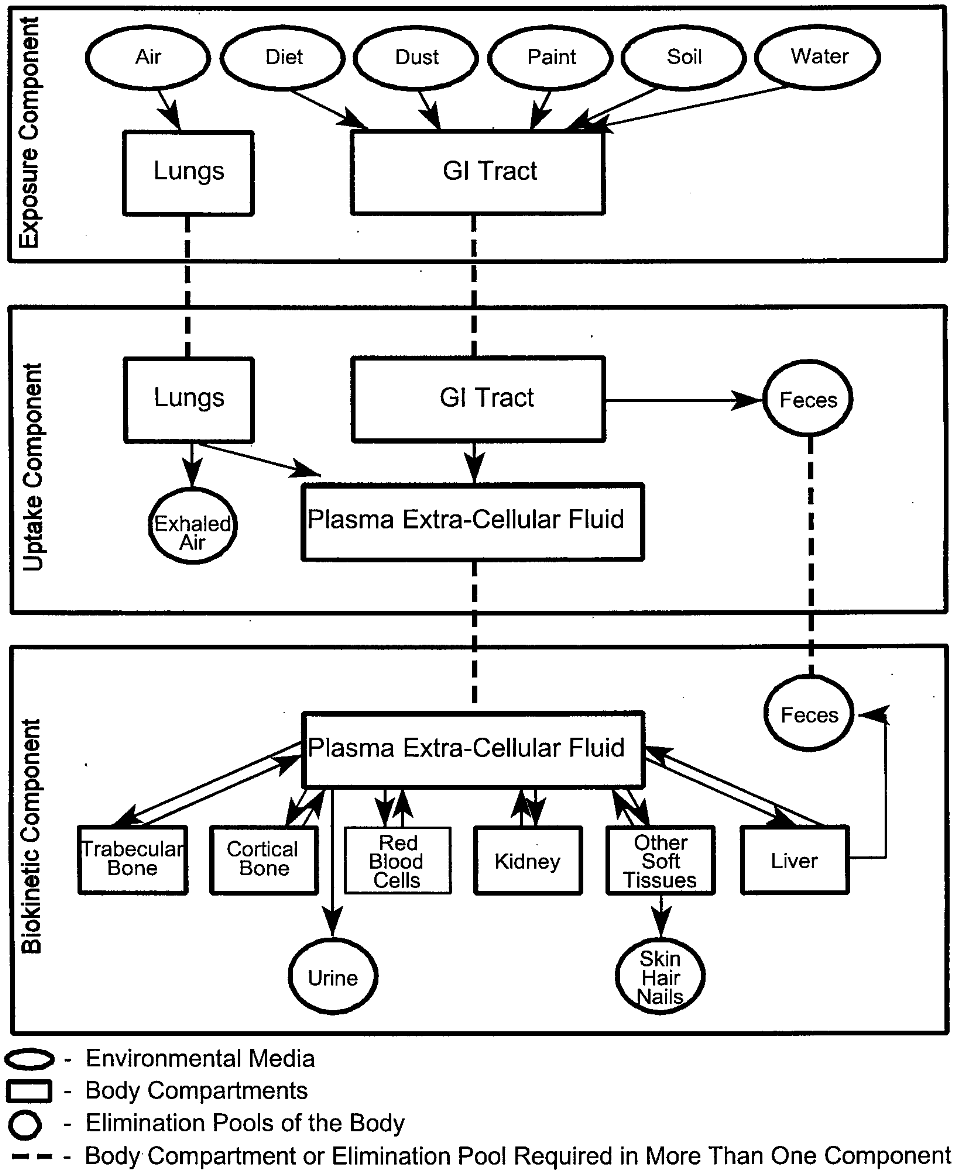


Figure 3: *Conceptual diagram of the movement of environmental lead into and through the human body*[68]

The foundation of the present IEUBK model is the construction of a detailed and thorough exposure scenario for children aged 0 to 84 months that can be adjusted to match the exposure of any child. The user starts with exposure information specific to these children and accepts generalized assumptions about any additional information required to complete the exposure scenario. The site-specific information usually consists of environmental media concentrations such as soil lead concentrations. The model inserts default values whenever site-specific information is not used. The default values (e.g., dietary lead concentrations and consumption values) are typical of a child's environment in the sense that they are broad-based estimates of the expected environment of a child. These default values are not necessarily appropriate for every site and should be reviewed by the user for every site-specific application.

This model uses standard age-weighted exposure parameters for consumption of food, drinking water, soil, and dust, and inhalation of air, matched with site-specific concentrations of lead in these media, to estimate exposure for the child. The model simulations represent chronic exposure and do not incorporate the variability in consumption patterns and media concentrations on a daily or seasonal basis. The model includes continuous growth of the child and simulates the changing environment of the child on a yearly basis. In theory, the exposure component of the model would apply to a single child or to any number of children with the same lead exposure scenario. With the proper substitution for media concentrations, the exposure component (but not the biokinetic component) would also apply to any other substance with sources and pathways of exposure similar to lead.[66]

3.1.1 Application of the IEUBK Model

The IEUBK model can be applied at several different scales of application. In simplest use of the model, a site is a spatial domain that is appropriate for remediation decisions, typically a residential yard with a single housing unit, or an equivalent area for multi-unit buildings or for undeveloped lots. The home and its surrounding yard is the basic unit for risk analysis because lead exposure for pre-school children commonly occurs within this domain.

An array of applications of the IEUBK model based on aggregating clusters of sites:

A: One location
  A1: one living unit, one child;
  A2: one living unit, more than one child;
  A3: more than one living unit, more than one child, homogeneous media concentrations;
B: Multiple locations, one neighborhood, homogeneous media concentrations
C: Multiple locations, one neighborhood, heterogeneous media concentrations;
D: Multiple locations, more than one neighborhood, heterogeneous media concentrations;

A single run of the IEUBK model is sufficient for categories A and B. A classification or disaggregation of the neighborhood into distinct exposure subgroups is required in categories C and D, with the possibility of different ingestion or absorption parameters for different neighborhoods in category D. Neighborhood-scale and community-scale risk estimation requires aggregating the risk estimates for individuals or subgroups. The differences between these levels are sketched in Figure 4.[66]

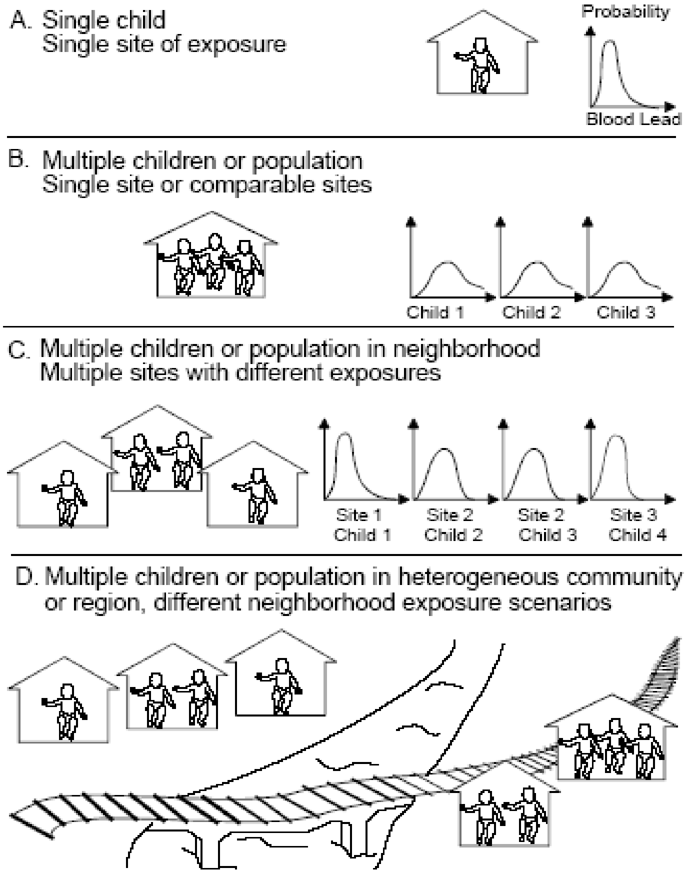


Figure 4: *Levels of application of the IEUBK Model* [66]

The model calculates the probability that a blood lead concentration derived from the model's specified parameters will exceed a level of concern specified by the user.

The model output in category A: Single child, single site of exposure, includes a blood lead concentration, a distribution of blood lead concentrations, and a probability of exceeding the blood lead level of concern. Since children in environments with the same lead exposure may have a range of blood lead concentrations, we describe the likely variability in blood lead for a child with a given exposure scenario by a probability distribution. The predicted blood lead concentration is the geometric mean of the distribution of blood lead concentrations that may occur for a typical child with the specified exposure scenario. Risk is calculated from this distribution as the probability that a hypothetical child living at this site, with the specified exposure scenario will have a blood lead concentration exceeding the blood lead level of concern. The upper tail of the probability distribution provides an estimate of the risk of exceeding blood lead level of concern.

The model output in category B: Multiple children, single site or equivalent sites of exposure, is the predicted blood lead concentration for each child as the geometric mean of the distribution of blood lead concentrations that may occur for each child with the specified exposure scenario. Risk is calculated by aggregating the calculated risk for each child as the percentage of hypothetical children living at this site or at these sites, with the specified exposure scenario, which will have a blood lead concentration exceeding the blood lead level of concern. The upper tail of the probability distribution represents the fraction of children exceeding the chosen blood lead level of concern in this case. The calculation is exactly the same as the single-child assessment, but there is an important shift in interpretation of the output.

The model output in C: Multiple children, multiple sites with different exposure, cannot be obtained by a single run of the IEUBK model. It is necessary to construct an exposure scenario for each distinct exposure subgroup in the population. For each child or exposure subgroup, risk is calculated in a single run of the IEUBK model with the specified exposure scenario. The risks for each exposure subgroup are aggregated across all subgroups, weighted by the number of children with that exposure scenario or by the percentage or likelihood of the exposure scenario. There is no one-step method by which neighborhood-scale risk estimation can be done using this version of the IEUBK model. The problem of risk estimation for children in a large community or a region is even more difficult when different subgroups of children may have very different exposure scenarios, including differences in behavior that affect ingestion, and differences in lead absorption due to behavioral or nutritional differences. A common misinterpretation of the IEUBK Model is that it predicts community geometric mean blood lead and the fraction of children at risk when the input is the mean or geometric mean of household-specific environmental lead concentrations. That misstep can be misleading, particularly when the environmental variables have a wide distribution among the neighborhoods of the community. This misinterpretation is especially dangerous for post-abatement settings intended to eliminate the higher exposures when there are multiple exposure media. A correct approach requires applying the model to each individual home or site using the lead concentrations seen at that site and combining these results as an aggregate of sites in several neighborhoods to form an estimate of community risk. A second useful approach is based on subdividing a community into neighborhoods and clusters of residence units with similar media lead concentrations.[66]

3.1.2 A site-specific exposure scenario

The use of the IEUBK model requires input data that are appropriate to the sites and subjects. For most assessments of lead-contaminated soils, the minimal site-specific data are the soil lead and indoor dust lead concentrations for the residential exposure unit. Additionally, it would be helpful to include estimates of specific exposures from diet, drinking water, air, maternal exposure, or other sources that could replace the default exposure parameters believed to be of concern at the particular site. There may be potentially important differences among sites, and predictions of blood lead values are expected to become more accurate as more site-specific data are added.[66]

3.1.3 Parameter Input

For better results it is necessary to use site specific data and to concentrate on smaller area. In that way exposure of a typical child living in that area is better defined. The IEUBK Model allows changes of practically any parameter, but also provides default values which are EPA-s best estimates for urban residents with no unusual lead exposures. The estimated blood lead levels with the default parameters represent best estimate of the blood lead "background" levels that cannot be avoided. Default values are provided for the convenience of the user, but these values may not be appropriate for specific applications. The user has the ultimate responsibility for justification of values used in the applications of the model. Most of the parameters will not need to be modified, but the user should be aware of them. Many default parameters in the model have only a minor effect on the results (i.e., a 10% change in the air lead concentration parameter will change blood lead levels by less than 1%), but some parameters may be more influential. The parameters and default values are described in following text.[66]

3.1.3.1 Air lead

The air lead concentration is set initially to a typical 1993 urban value of 0.1 μg/m^3. Air lead concentrations measured in some cities in following years showed that urban air lead concentrations are still around that value.[69] The time spent outdoors and ventilation rate are assumed to depend on the child's age. From these parameters a time-weighted air lead intake is calculated; 32% of that intake is absorbed into the child's blood. All parameters may be changed by user. When adequate monitoring data for outside air concentrations exist, these should be used instead of default values. Age specific default values for child ventilation rates and outdoor time were established by EPA. Children who exercise more than average will have a greater intake and those who are very inactive will have a lower ventilation rate than default values are. The outdoor air lead concentration provides a large part of the total air lead exposure, because the indoor air lead concentration is typically only about 30% (default value 0.03 μg/m^3) of the outdoor.[66]

3.1.3.2 Dietary lead

The daily dietary lead intake values for each age apply to a typical U.S. child in a typical setting in the United States after 1990. Data assembled from a variety of sources, including Market Basket Surveys[70] and representing changes in consumer behavior over time, were used to construct dietary lead intake estimates. These dietary lead values may be changed. During the period 1982-1989 there was a distinct reduction in food lead generally attributed to the replacement of lead-soldered cans and the removal of lead from gasoline. Since 1990, food lead in U.S. supermarket food has remained relatively constant. Default value for dietary lead intake of 3 years old children is 6.24 μg/day. Studies conducted in Europe showed that dietary lead intake could be a bit lower on average, but higher in very industrial areas.[71,72] Dietary lead intake can be bigger in some lead contaminated areas if lead locally produced food is important part of the diet. In this case the default values may be altered. Seasonal effects are not a factor here since the IEUBK Model uses annual values for dietary exposure parameters. If the site-specific dietary levels are not available, it is recommended that the default values are used.

3.1.3.3 Drinking water lead

The water lead concentration is set initially to a typical 1990 urban value of 4 μg/l.[73] The age-specific ingestion of tap water values are used. Concentration and consumption may be modified by entering new values, if appropriate data exist. In drinking water there can be more lead if supply system pipes contain lead. In this case one must be aware of that when changing input values. Water lead concentrations in first draw samples would be higher than lead concentrations in flushed samples. For children receiving formula reconstituted with tap water, consumption of tap water is much higher, so in an assessment addressing risks from lead in drinking water, the exposure to infants consuming reconstituted formula requires specific attention. [66]

3.1.3.4 Soil and dust lead

The soil and dust lead concentrations are set initially to a value of 200 μg/g, but it is recommended to use site specific data. The age-specific ingestion intake of soil and dust was estimated from the EPAs paper on Exposure Assessment Methodology and Validation for the first version of the UBK model. The default value for total intake of soil and dust depends on age, and ranges from 85 to 135 mg/day. These values represent a kind of central value within the range of values seen in different studies.[74] Concentration and intake rates for soil and dust may be changed by the user.

It is best to get site specific household dust data. If that has not been done, then concentration can be estimated by calculation. The multiple-source option on the dust entry field allows the user to use information about the contribution of soil lead, air lead, and other sources to household dust lead. There are three options to enter data. The first represents the fraction of the soil lead concentration that contributes to the concentration of lead in household dust. The default is 70% and is appropriate to residences in which loose particles of surface soil are readily transported into the house. The second represents the contribution to household dust from the deposition of airborne lead. The current default value is 100 μg/g lead in house dust for each μg

Pb/m^3 air. This is added to dust concentration calculated from soil lead concentration. The third option allows the user to add other sources. The user may assign both the concentration and percentage of dust intake to baseline household dust, secondary occupational dust, dust at school, daycare, or second home, and the exposure to lead in dust from household paint measured as a percentage of total dust ingestion and its concentration. The default dust lead concentration for the alternative indoor dust entry is 100% of household dust at 150 μg/g. If the Alternative Source Analysis is not used, then the default dust contribution consists of 70% of the soil concentration plus 100 times the air lead concentration. For default conditions, the total dust lead concentration equals 150 μg/g. If non-residential exposures to soil/dust are important, the user may enter these as well. The combined soil/dust ingestion rate (grams total soil + dust per day) can also be changed.

A relationship between dust lead concentration, soil lead concentration and air lead concentration is shown by following equation.

$$PbD = \beta_O + \beta_S PbS + \beta_A PbA \qquad \text{(Equation 1)}$$

In this equation house dust lead concentration is denoted as PbD, a soil lead concentration in mg /kg is denoted as PbS and air lead concentration is denoted as PbA. Factors β_s and β_A represent fractions of the soil lead and of the air lead that contribute to house dust lead concentration, and factor β_O represent dust from unidentified sources.

Using of the default assumption that 45% of the total dust intake is derived from soil is recommended. The ratio of soil intake to dust intake is not simply proportional to the ratio of the number of waking hours that the child spends outdoors versus indoors. Children spend only 15 to 30% of their waking hours playing outside but are more likely to be in contact with bare soil areas, in locations with large amounts of accessible loose particles, and are likely to wash their hands less often than when they are indoors. The default 45/55 ratio in the model represents EPA-s best judgment of a properly weighted ratio for this parameter. The distinction between soil and dust is important because there is some indication that even if soil lead is the principal source of dust lead, there may be chemical or physical differences between soil and dust that may affect bioavailability. In cases where house dust differs significantly from soil derived dust the soil/dust ratio becomes important. One example might be the presence of interior lead-based paint. In this case the parameter can be effective in separating soil derived dust and paint derived dust into two components where both the amount ingested and percent absorbed can be correctly input into the model. There is some evidence that the soil intake is very responsive to exogenous factors, such as weather.[75] The lowest soil and dust intakes at daycare centers should occurre in rainy weather, when the children had the least amount of outdoor activity, also long winters with heavy snow fall can have large influence.[66]

3.1.3.5 Alternate source

The default daily lead intake value for each age for alternate sources is set to 0 μg Pb/day. The user can add sources which are not included in other menus. (i.e., direct ingestion of lead-based paint, cosmetics or home remedies).[66]

3.1.4 Bioavailability of lead in food, drinking water, soil, and dust

Bioavailability or absorption of intake from the gut or lung into the blood is a key element in relating external exposure to body burden. Lead intake from media with low bioavailability poses much less of a hazard than does the same intake from media with high bioavailability. The bioavailability of lead from normal infant diet is known to be very high[27,28,76] with at least 40 to 50% of the dietary lead intake passing into the child's blood.

The model calculates lead absorption from the gut as a function of two components. The passive component does not depend on lead concentration in the gut and is not saturable. The other is active component which may become saturated when the total concentration of lead in the gut is sufficiently large, which is a kinetically non-linear absorption mechanism. Model allows the user to specify the parameters for intake from soil, dust, drinking water, diet, and alternate sources. The total absorption percentage is the sum of the passive and active absorption components. The default value of absorption for alternate sources is 0%.

The total absorption from any medium is then divided into two components, and the user specifies a small fraction of the total absorption percent for the passive or nonsaturable (i.e., high-dose) component. The default is 20% of the total available for absorption. The percentage absorption in the larger saturable component is the remainder of the total available for absorption. For example, with a dietary lead intake of 50%, the absorption fraction for the passive component is 20% of 50%, or 10% of dietary lead intake, and the saturable component is 80% of 50%, or 40% of dietary lead intake.

The current assumption in the IEUBK Model is that 30% of dust and soil lead intake is absorbed into the blood. This is assumed to be partitioned into a nonsaturable component of 6% and a saturable component of 24%.

Relative bioavailability of lead in soil compared to water and food is about 60%. Recent studies showed that the bioavailability of lead in different samples of soil may be very different, what highlights the importance of obtaining and applying reliable bioavailability data to site-specific samples in order help to improve risk assessments for lead exposure.[77]

3.1.5 Maternal-Fetal lead exposure

The lead is transferred from the mother to the fetus in utero. To calculate the lead concentration in the blood of the newborn one must enter the maternal blood lead value at birth (default = 25 µg/l). The IEUBK model assumes that the infant's blood lead at birth is a fraction of the maternal blood lead level. The amount of lead in the blood of the newborn child is calculated so as to be consistent with concentration ratios of lead in tissue observed in autopsies of newborn infants. [78]

3.1.6 Computation menu

There are three different options of running the calculations in the IEUBK model:

- Run a Single Simulation of the Model;
- Run Multiple Simulations of the Model for a Range of Media Lead;
- Batch Mode Multiple Simulation Runs Using Input Data Files.

When running a single simulation of the Model option only the currently loaded parameter set is used. The user may view or change the time step for the numerical iteration. The default is four hours. An output option allows plotting of results and calculation of probability of elevated blood lead.

“Run Multiple Simulations of the Model for a Range of Media Lead” uses only the currently loaded parameter set, except that it repeats the run for each new value of a medium concentration or intake. The user may view or change the time step for the numerical iteration during the run step. Only one medium can be changed in each use.

Batch Mode Multiple Simulation option uses the currently loaded default parameter set, but repeats the run for using the new values for the five exposure parameters (soil concentration, dust concentration, drinking water concentration, air concentration and alternate source consumption) for each child in the data set. The input data are entered one line at a time from a data set with a specified list of input variables. These must be created by the user. The batch mode option can be used to perform statistical analyses of simulated community blood lead distributions, even without observed blood lead levels. However, this option is even more useful if blood lead data from a well-conducted study are available for model comparisons using statistical tests.[66]

When calculating the proportion of children with elevated blood lead levels beside a cutoff value (default ≥ 100 μg/l) user should also choose value for the Geometric Standard Deviation (GSD). GSD is a measure of the relative variability in blood lead of a child of a specified age, or children from a hypothetical population, whose lead exposures in a specified dwelling are known. The default value for GSD is set to 1.6 and it is recommended to use this value, although GSD values between 1.3 and 1.8 are allowed by the model.

3.2 Sampling and analyzing methods

3.2.1 Air

Sampling of air samples and analyses were carried out by ERICO. There were 4 sampling locations. Sampling started on 9 January and finished on 27 December 2002. Air pumps were located at a height on which a human of average height inhales air. The samples were pumped through membrane filters (polycarbonate, with a diameter of 0.45 μm). The filters were weighed before and after sampling, the difference in mass meaning the mass of aerosols. Mettler Toledo AT 201 weighing machine was used to determine sample mass. An average annual concentration of aerosols was calculated by dividing the mass with volume of air pumped through the filter. Analyses were carried out in January 2003 in the ERICOs laboratory. Samples were taken and analyses were carried out according to demands of SIST EN ISO 17025 standard. The samples were prepared by a microwave wet ashing of filters in acid mixture ($HCl:HNO_3$ = 3:1, HF) in microwawe apparatus Milestone Ethos Plus. ICP-MS (Inductively coupled plasma mass spectroscopy) method was used for prepared samples analyses. The instrument HP4500 was used. Concentrations were determined using the calibration curve method. The certified reference standard material SRM NIST 1640 (trace elements in natural water) was used to assure procedure accuracy. The measurement uncertainty of preparing and analyzing samples was evaluated to be 10%.

3.2.2 Soil and dust

Soil sampling and sample preparation were carried out in accordance with demands put down in the Regulation on the Operation Monitoring of the Input of Dangerous Substances and Plant Nutrients into the Soil (Official Journal RS, 55/97)[79] and in accordance with ISO standards 10381/1-6 (sampling) and ISO 11464 (preparation). The diagonal system of soil sampling in a depth 0 to 20 cm was used. The samples were dried on temperature of 36°C. The dry samples were homogenized and screened through the plastic sieve (2 mm). For the elemental analyses, the samples were grinded to a size of 150 μm. The samples were disconnected with the aqua regia in the microwave apparatus CEM MSP 1000. Lead concentration was defined by AAS (atomic absorption spectroscopy) with electrochemical atomization. Perkin Elmer SIMAA 6000 apparatus was used. The standard reference material NIST SRM 2710 (Montana soil) with a certified value of 5532 ± 80 mg Pb /kg was used for the method validation. The results of laboratory analyses were considered satisfying if the measured values of standard reference material were within 10% of the certified value (Table 1).

Table 1: *Measurements of the certified reference material NIST SRM 2710*

Declared value		*5532± 80 mg/kg*	
Maximal acceptable value		*6085 mg/kg*	
Minimal acceptable value		*4979 mg/kg*	
Measured values (mg/l)			
1.	*5730*	*4.*	*5610*
2.	*5620*	*5.*	*5720*
3.	*5130*	*6.*	*5510*
Average± SD		*5553 ± 223 mg/kg*	

Indoor dust is a fraction of surface dust (urban sediment) closely connected to places where inhabitants spend most of their time. It has a heterogenic structure, which is influenced by habits and activities of the inhabitants. Indoor dust samples were collected from household vacuum cleaner bags. The samples from three different households in the neighborhood were joined in one combined sample, analyzed on lead concentration. The samples were dried on air and also in a ventilation oven at 35°C. After the removal of larger pieces, the samples were divided in half. One half was prepared for analyses and the other was put into the archive. The samples were screened through the stainless steel sieve (< 0,125 mm). The samples were grinded using an achat mill if necessary. Lead content was determined by ICP-MS (inductively coupled plasma mass spectrometry) method in which 0.5 g of a sample is diluted in 10 ml acid mixture ($HClO_4$, HNO_3, HCl and HF) at 200°C. The standard reference material NIST SRM 1643d (trace elements in water) with certified value 18.15 ± 0.64 μg Pb /l was used for the method validation. The results of laboratory analyses were considered satisfying if measured values of standard reference material were within 10% of the certified value (Table 2).

Table 2: *Measurements of the certified reference material NIST SRM 1643d*

Declared value		*18.15± 0.64 μg/l*	
Maximal acceptable value		*16.34 mg/kg*	
Minimal acceptable value		*19.97 mg/kg*	
Measured values (mg/l)			
1.	*18.12*	*4.*	*19.54*
2.	*16.95*	*5.*	*18.77*
3.	*17.54*	*6.*	*18.51*
Average± SD		*18.24 ± 0.92 μg/l*	

3.2.3 Drinking water

Data for lead in drinking water was taken from the archives of the Regional Institute of Public Health Ravne na Koroškem. Water samples were collected from the pipes outlets of the end users after flushing. Analyses were carried out in the laboratory of the Regional Institute of Public Health Maribor. The analyses were carried out according to ISO 17294-2:2003 (Water quality-Application of inductively coupled plasma mass spectrometry (ICP-MS)–Part 2: Determination of 62 elements. ICP-MS Perkin-Elmer apparatus was employed for the analyses. The method is validated and accredited. The result of the measurement is the sum of the isotopes Pb206, Pb207 and Pb208. The working range of the method is 1-1000 μg/l, the limit of detection (LOD) is 0.005 μg/l and the limit of quantitation (LOQ) is 1 μg/l. LOD is defined as three times the standard deviation of the blank measurement. Measurement uncertainty for lead amounts to 7%. It is calculated on the basis of repeatability at LOQ level, recovery and precision. The recovery is calculated from measurements of certified reference material (NIST 1640 – trace elements in natural water with a declared value of 27.89 ± 0.14 μg/kg Pb; density – 1,0015± 0,0005 g/cm^3) in every series of measurement, and the precision is derived from standard reference material measurements at the validation of the method (measured value of NIST 1640 - 28,10 μg/l, recovery 101 %).

3.2.4 Blood lead

For blood lead analyses, capillary blood samples of three-year old children were collected when attending their regularly systematic health check. The samples were collected at the local health stations. They were collected in the laboratory by trained specialists. A strict procedure was followed to minimize the likelihood of contamination. The child's hands were washed with soap and then dried with a clean towel. Once washed, special care was taken to prevent a finger coming into contact with any surface, including the child's other fingers. The finger to be punctured was massaged to increase circulation before being punctured with the lancet. The ball of the finger to be punctured was cleaned with an alcohol swab and dried prior to the puncture. After the puncture with sterile lancet was performed, the first droop of blood was removed and blood was collected in a test tube. The test tubes containing EDTA were used. The collected samples were agitated to mix the anticoagulant through the blood. Materials used in the collection procedure were all lead-free. The samples were stored frozen until the analyses were conducted. Before the analyses, the samples were diluted with 0.25 % ascorbic acid and 1% Triton X-100 in 1:5 ratio. The blood samples were analyzed directly with GFAAS (graphite furnace atomic absorption spectroscopy). To achieve the ashing of organic matrix, air was initiated into a graphite furnace in a disconnection phase instead of argon. Lead absorbance was measured at 283.3 nm. Deuterium was used for background correction. Every blood lead sample was measured in two parallels and the result was presented as an average of both measurements. A standard deviation was calculated from the results of ten measurements of different parallel samples. The reliability of the results was satisfactory if the difference between two parallel measurements did not exceed twofold standard deviation, otherwise the measurements were repeated. To assure the accuracy of measurement, certified reference material BCR CRM 194 with declared value of 126± 4μg/l Pb was used. It was prepared in the same way as the blood samples. The measurement results of a reference material showed a satisfactory agreement with the declared value (Table 3).

Table 3: *Measurements of the certified reference material BCR CRM 194*

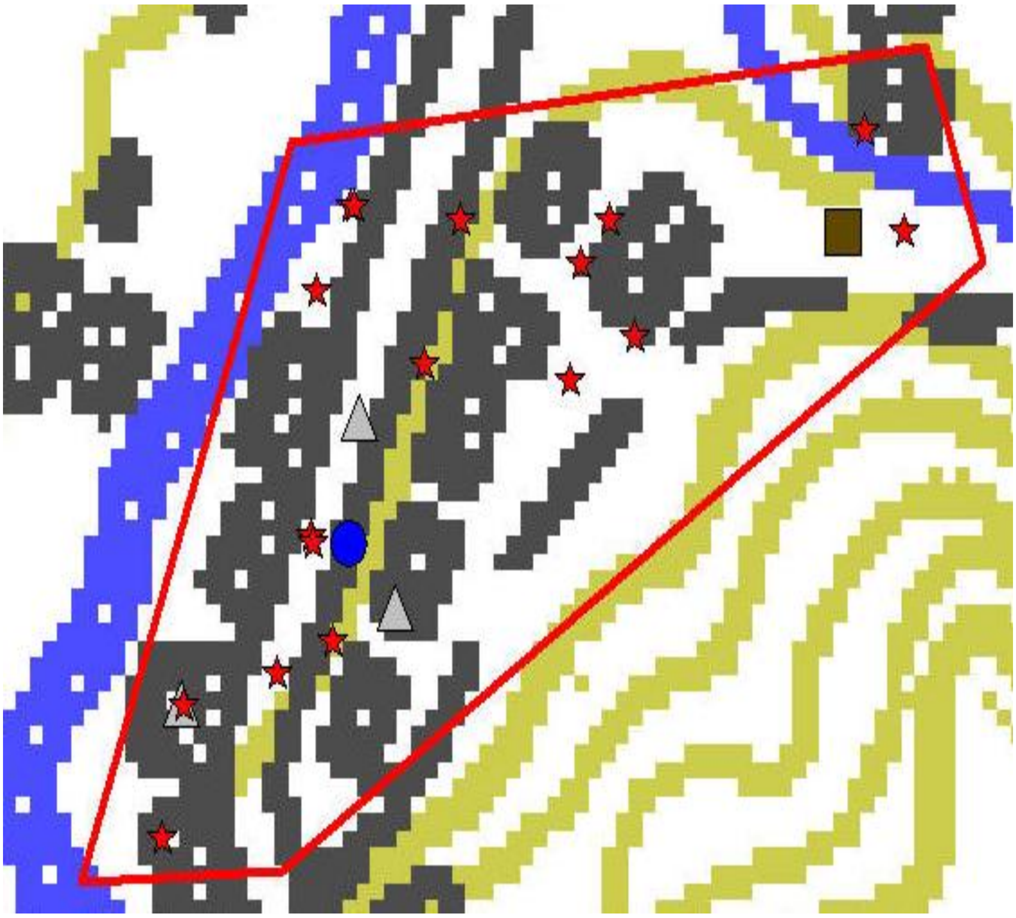
Measured values (mg/l)			
1.	*130*	*5.*	*130*
2.	*128*	*6.*	*145*
3.	*140*	*7.*	*103*
4.	*111*	*8.*	*118*
Average± SD		*125.6 ± 14.2 μg/l*	
Declared value		*126 ± 4 μg/l*	

The method has the following characteristics: detection limit is 10 μg/l Pb, the real sample repeatability adds up to 10% and combined uncertainty is 15%. The same methodology carried out by the same laboratory was used in ERICOs study and in the project “Living with lead”, thus the data from both studies could be joined for the purposes of our study.

3.3 Calculations

The Integrated Exposure Uptake Biokinetic Model for Lead in Children, Windows® version (IEUBKwin v 1.0 build 264) was used for calculation. The software is available on EPA website (http://www.epa.gov/superfund/lead/products.htm) and can be downloaded free of charge. We downloaded our version on 3 September 2007.

At first, the data was mapped on the map of the Koroška region. The data was mapped in the Gauss-Krüger coordinate system according to a sampling place (environmental factors) or address (children). Map Info 5.0 software was used. In the following step, groups were formed in which environment pollution data and lead burden of children from the same geographic area (neighborhood) were merged. (Figure 5)



Marked locations for: ★Child ▲ Dust ■ Soil ● Air

Figure 5: *Example of a group which includes environmental and blood burden data from the same area*

The environmental lead burden data for each group was used in the IEUBK model. A batch file was created in which every group was described by site specific concentration for soil and dust lead and also for air and drinking water lead, where the data was available. For other parameters, the IEUBK default values were used.

The calculations to predict the geometric mean blood lead concentration in hypothetical population of children and to calculate the hypothetical proportion of children with elevated blood lead level (≥100 µg/l) were performed. The calculations with the IEUBK model were also carried out for each separate group. The hypothetical proportion of children with elevated blood lead level was calculated with a default value for geometric standard deviation. The results from these calculations were compared with the empirical data (geometric mean, proportion of children with BLL ≥100 µg/l) on blood lead burden in children from the same group. The calculations with empirical data are described by the following equations:

$$GM = \sqrt[N]{BLL_1 * BLL_2 * ... * BLL_N} \qquad \text{(Equation 2)}$$

GM – geometric mean, N – number of children, BLL – blood lead level

$$P = \frac{N_{(BLL>100\mu g/l)}}{N_{(all)}} \qquad \text{(Equation 3)}$$

P - proportion of children with elevated levels, N – number of children

Microsoft Excel and SPSS 8.0 for Windows software packages were used for calculations with empirical data on the children's blood lead burden. To predict proportion of children with elevated blood lead levels for the whole area under study, predicted proportions for group with number of children in that same group were multiplied. Products for all groups were added up and the sum was divided by the number of children included in all groups.

Another approach was also employed in which a net of 1000 by 1000 meters squares was created over the map of the entire area of concern. Data from the same square represented each group. Since there were different sampling points for same environmental factors in some groups, calculations with the IEUBK model using average values of measured values were performed. The results were compared with empirical data. This approach is not as suitable for the IEUBK model as the first one, but it was decided to test it anyway.

Some additional simple comparisons and calculations were conducted to asses if the children living in a more contaminated area have higher risk for elevated blood lead levels.

3.4 Statistical methods

Our hypothesis was that the IEUBK model will act well when using data on environmental lead pollution for the Upper Meža Valley and that predicted values for geometric mean blood lead level and the proportion of children with elevated blood lead levels would be in satisfying agreement with observed values. To test it, two different methods were chosen. For smaller, better defined areas (groups, locations), 95 % confidence intervals for each predicted and observed value were calculated. A 95% confidence interval around the mean was calculated according to equation 4 and a 95% confidence interval for proportion of children with elevated blood lead levels was calculated according to equation 5. Both values (predicted and empirical) and corresponding confidence intervals were compared.

$$CI(95\%) = \exp\left(\ln(GM) \pm t_{0.975} * \frac{\ln(GSD)}{\sqrt{N-1}} \right) \qquad \textit{(Equation 4)}$$

GM – geometric mean, GSD – geometric standard deviation, N- number of children

$$p = \frac{P + \frac{1.96^2}{2N} \pm 1.96\sqrt{\frac{P(1-P)}{N} + \frac{1.96^2}{4N^2}}}{1 + \frac{1.96^2}{N}} \qquad \textit{(Equation 5)}$$

P - proportion of children with elevated levels, N – number of children

McNemar's test was used (Table 4) for the purposes of testing the hypothesis for the whole area. An empirical sample of children was compared to a hypothetical sample of children formed on basis of the IEUBK models prediction. Observed cases with blood lead level equal to or over 100 μg/l (observed value) were compared with hypothetical cases with elevated blood lead levels. For purpose of the test, it was decided that the children from groups in which the IEUBK model predicted at least a 66.7% (cutpoint) chance of elevated blood lead level will represent the cases with elevated blood lead levels, while children from other groups will not. The differences were considered statistically significant if p value was less than 0.05.

Table 4: *McNemar's test calculation*

		Observed ≥100 μg/l	
		Yes = 1	*No = 0*
3 *Predicted ≥ 100 μg/l*	*Yes = 1*	*A*	*B*
	No = 0	*C*	*D*
		$\chi^2 = \frac{(B-C)^2}{(B+C)}$	

To test if there are important differences in blood lead burden of children according to sex and community of residence, a chi square test has been applied. (Table 5) The differences were considered statistically significant if p value was less than 0.05.

Table 5: *Chi square calculation*

Number of children	$N_{(BLL \geq 100\ \mu g/l)}$	$N_{(BLL < 100\ \mu g/l)}$	$N_{(all)}$
Observed	O_1	O_2	$N_{(all)} = O_1 + O_2$
Expected	E_1	E_2	$N_{(all)} = E_1 + E_2$
χ^2	$R_1 = \frac{(O_1 - E_1)^2}{E_1}$	$R_2 = \frac{(O_2 - E_2)^2}{E_2}$	$\chi^2 = R_1 + R_2$

4 RESULTS

4.1 Environmental data

For calculations with the IEUBK Model, data on air, soil and house dust lead burden from The Comparative study of the Environment Pollution in the Upper Meža Valley between 1989 and 2001 were used. Data on air quality, house dust and soil were employed. The data used in calculations is presented in the following tables (Tables 6-8).

Table 6: *Air lead concentrations in the Upper Meža Valley area in year 2002* [46]

Mark (location)	*Conc. Pb (μg/m3)*
AirZ	*0,072*
AirR	*0,083*
AirM	*0,054*
AirC	*0,076*

Table 7: *Lead concentrations in house dust in the Upper Meža Valley area in year 2002*[46]

Mark (location)	*Pb (mg/kg)*	*Mark (location)*	*Pb (mg/kg)*	*Mark (location)*	*Pb (mg/kg)*
MS1	*787*	*MS6*	*1024*	*CS1*	*1426*
MS2	*839*	*MS7*	*1185*	*CS2*	*1092*
MS3	*524*	*MS8*	*1287*	*CS3*	*1114*
MS4	*787*	*ZS1*	*2277*	*CS4*	*1087*
MS5	*737*	*ZS2*	*2126*	*CS5*	*724*
Average lead concentration [mg/kg]					*1134 ± 496*

Table 8: *Lead concentrations in garden soil in the Upper Meža Valley area in year 2002 (mg/kg dry soil)*[46]

Mark (location)	*Pb (mg/kg)*	*Mark (location)*	*Pb (mg/kg)*
M1	*1410*	*Č3*	*1190*
M2	*2830*	*Č4*	*1890*
M3	*898*	*Č5*	*925*
M4	*1030*	*Č6*	*1950*
M5	*597*	*Č7*	*897*
M6	*593*	*Č8*	*734*
M7	*947*	*Č9*	*1710*
M8	*936*	*Č10*	*511*
M9	*573*	*Č11*	*811*
Č1	*3350*	*Smelter*	*24500*
Č2	*4470*	*Control location*	*37.8*

Figures 6 and 7 show locations where samples of air, soil and dust were collected.

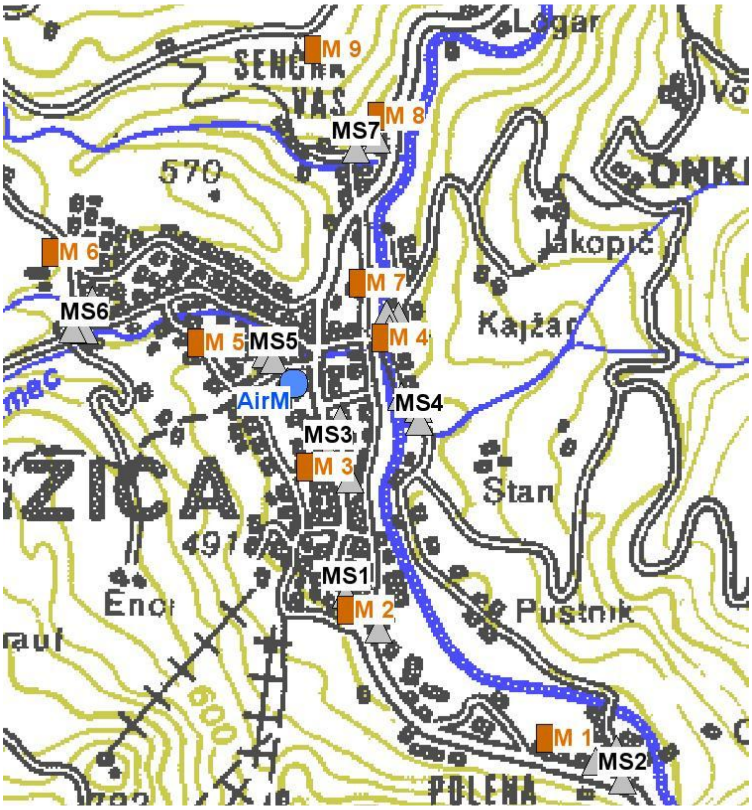


Figure 6: *Locations of sampling points for air, soil and house dust in the Mežica community*

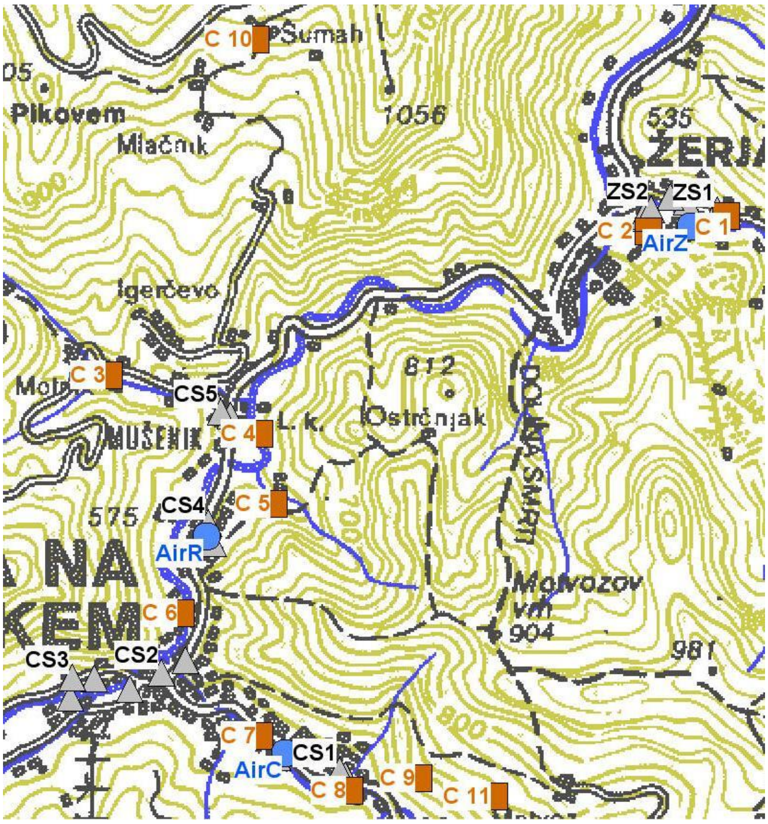


Figure 7: *Locations of sampling points for air, soil and house dust in the Črna na Koroškem community*

4.2 Drinking water data

For lead concentrations in drinking water, the data from the archives of the Regional Institute of Public Health Ravne na Koroškem was used. The results of water samples analyses from past years show that drinking water in the area is not contaminated with lead, with the exception of a part of a water supply system Črna na Koroškem, a water supply zone Center-Rudarjevo where lead was occasionally present. Average concentration of lead in water for the first half of year 2006 was used when drinking water was regularly checked for lead content (Table 9). For lead concentration in drinking water from other supply systems, a model default value was considered.

Table 9**:** *Lead concentration in drinking water from water supply zone Center – Rudarjevo in year 2006*

Date	*Conc. Pb (µg/l)*
9.1.2006	*3.5*
6.2.2006	*14.0*
6.3.2006	*8.7*
10.4.2006	*7.4*
average	*8.4± 4,3*

4.3 Blood burden data

The combined data on children lead blood burden from the Comparative study of the environment pollution in the Upper Meža Valley between 1989 and 2001 and from the project “Living with Lead”, part of which was also the testing of blood samples of three-year old children, were taken into consideration. 17 three-year old children were included from the first study. The blood samples were taken in year 2002. From the second study, 179 children were incorporated. The blood samples were taken in a four-year period between 2004 and 2007. In both studies, blood capillary samples were collected on location and then analyzed in the laboratory of the Regional Public Health Institute Celje. Out of 196 children, 97 came from the Mežica community and 99 from the Črna na Koroškem community, 105 were boys and 91 were girls.

Measured blood lead levels were between 10 and 500 µg/l (Figure 8). The analysis revealed that 54.6 % (107 of 196) of the examined children had blood levels equal to or greater than 100 µg/ l. An average blood lead level was 124.1 µg/l, geometric mean was 95.8 µg/l, median was 104 µg/l and mode was 110 µg/l.

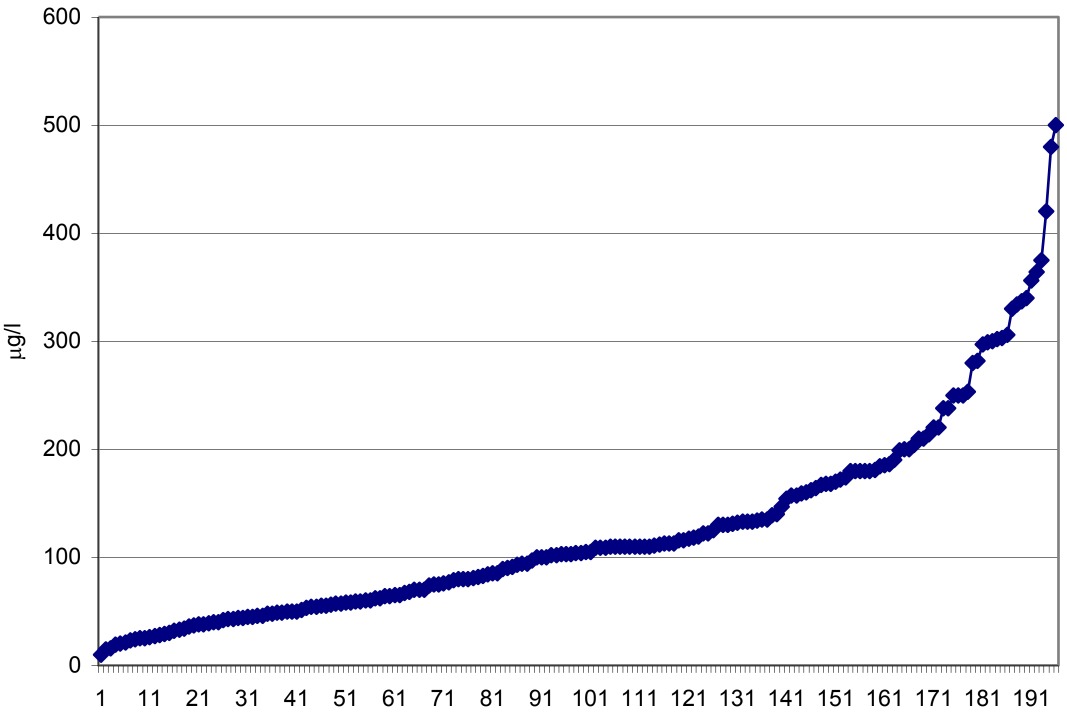


Figure 8*: Blood lead levels in three years old children from the Upper Meža Valley (sampling period 2001 –2002 and 2004-2007)*

Geometric mean blood lead value for the boys (105.9 µg/l) was higher than for the girls (85.4 µg/l) (Table 10). The analyses indicated elevated blood lead levels in 62.9% of the boys and in 45.1% of the girls. To confirm that the values of blood lead burden for the boys are more likely to exceed the level of concern (≥100 µg/l) than for the girls, a chi-square test was used. The result shows (Table 11) that the difference is statistically significant (p=0.013; d.f.=1). There was a larger proportion of the boys exceeding blood lead level of concern (≥100 µg/l), but extreme values over 400 µg/l were all observed in the girls (Figure 9).

Table 10**:** *Geometric mean, arithmetic mean, lowest and highest blood lead levels of 3-year-old children from the Upper Meža Valley according to sex, (sampling period 2001 – 2002 and 2004-2007)*

	N	*Geomean BLL (µg/l)*	*Average BLL (µg/l)*	*Min BLL (µg/l)*	*MAX BLL (µg/l)*
Male	*105*	*105.9*	*130.6*	*15*	*375*
Female	*91*	*85.4*	*116.7*	*10*	*500*
Total	*196*	*95.8*	*124.2*	*10*	*500*

Table 11: *Percentage of three years old children from the Upper Meža Valley with elevated blood lead levels (≥ 100 µg/l) according to sex, (chi-square test)*

	Male		*Female*		χ^2
	N	*%*	*N*	*%*	
< 100 µg/l	39	*37.1*	50	*54.9*	
≥ 100 µg/l	66	*62.9*	41	*45.1*	
Total	105	*100*	91	*100*	*6.23*

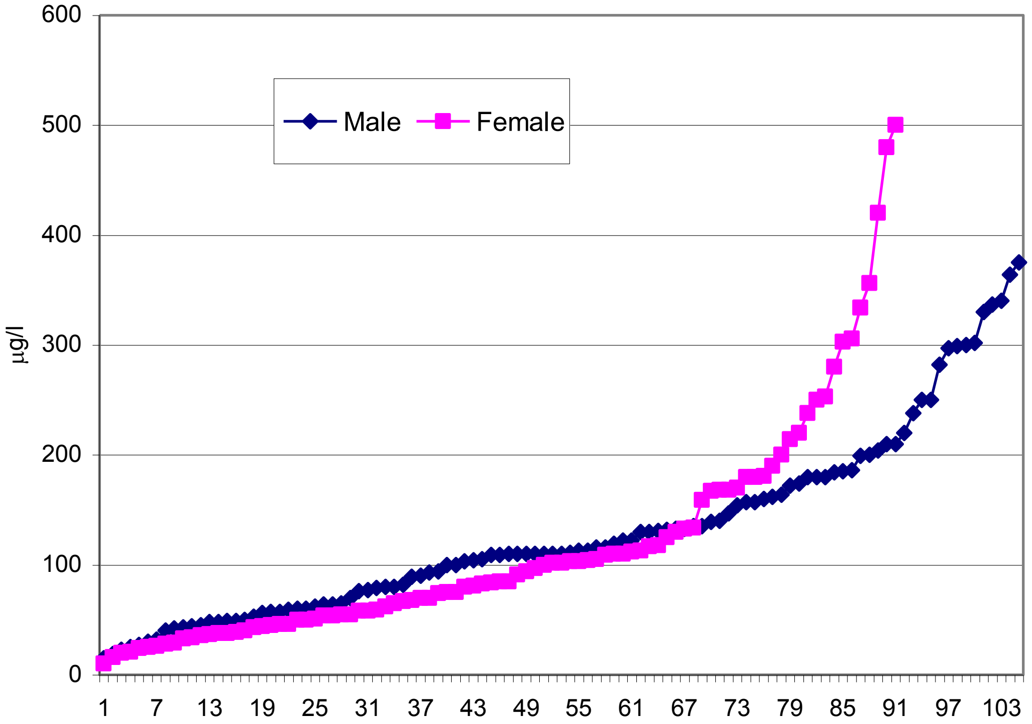


Figure 9: *Blood lead levels in three years old children from the Upper Meža Valley according to sex, (sampling period 2001 – 2002 and 2004-2007)*

Geometric mean blood lead level and proportion of children with elevated blood lead levels were higher for the Črna na Koroškem community than for the Mežica community (Table 12, Figure 10). To confirm that blood lead levels for the children from the Črna na Koroškem community are more likely to exceed the level of concern (≥100 µg/l) than for for the children from the Mežica community, a chi-square test was employed. The result shows (Table 13) that the difference is statistically significant (p=0.00002; d.f.=1).

Table 12: *Geometric mean, arithmetic mean, lowest and highest blood lead levels of 3-year-old children from the Upper Meža Valley according to community of residence,(sampling period 2001 – 2002 and 2004-2007)*

	N	*Geomean BB (μg/l)*	*Average BB (μg/l)*	*Min BB (μg/l)*	*MAX BB (μg/l)*
Črna	*99*	*120.4*	*150.5*	*15*	*500*
Mežica	*97*	*75.9*	*97.2*	*10*	*356*
Total	*196*	*95.8*	*124.1*	*10*	*500*

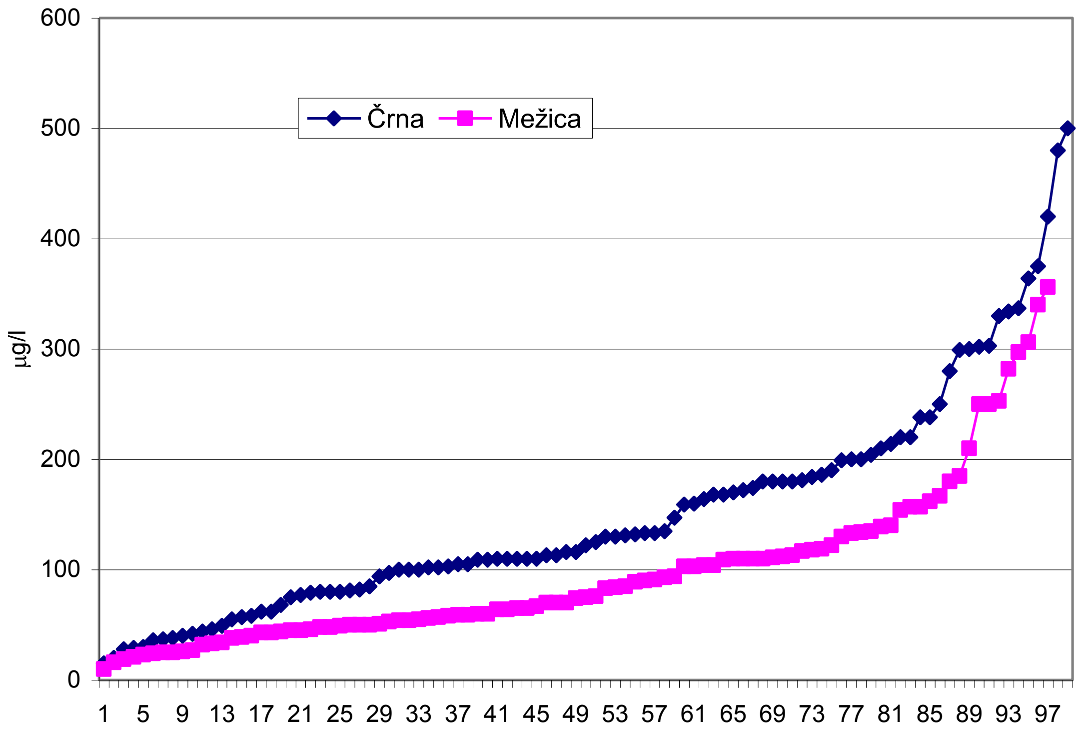


Figure 10: *Blood lead levels in three years old children from the Upper Meža Valley according to community of residence*

Table 13: *Percentage of three years old children from the Upper Meža Valley with elevated blood lead levels (≥ 100 µg/l) according to community of residence,(chi-square test)*

	Črna		*Mežica*		χ^2
	N	*%*	*N*	*%*	
< 100 μg/l	30	*30.3*	59	*60.8*	
≥ 100 μg/l	69	*69.7*	38	*39.2*	
Total	99	*100*	97	*100*	*18.41*

4.4 Comparison of the IEUBK model predictions with empirical data (groups)

To compare calculations of the IEUBK model with the empirical data, groups were merged in a way to incorporate data on environmental lead concentration and blood lead burden in children living near the sampling points for environmental media. Since sampling points for environmental media were not equally distributed in the area, some groups cover a larger area then others. 12 groups were formed in which 126 tested children were included. Other children were not incorporated in any group as they live in locations to far from the sampling points for environmental media. For each group, data on environmental lead concentrations was put into the IEUBK model and the calculation was carried out. Site specific data for soil, dust, air and drinking water lead burden was used with other default values from the IEUBK model. Predicted blood lead geometric mean and probability that the chosen blood lead level of concern (≥100 μg/l) will be exceeded were compared with geometric mean and the proportion of children with blood lead level ≥ 100 μg/l was calculated from empirical data. Group locations are presented in figures 11 and 12.

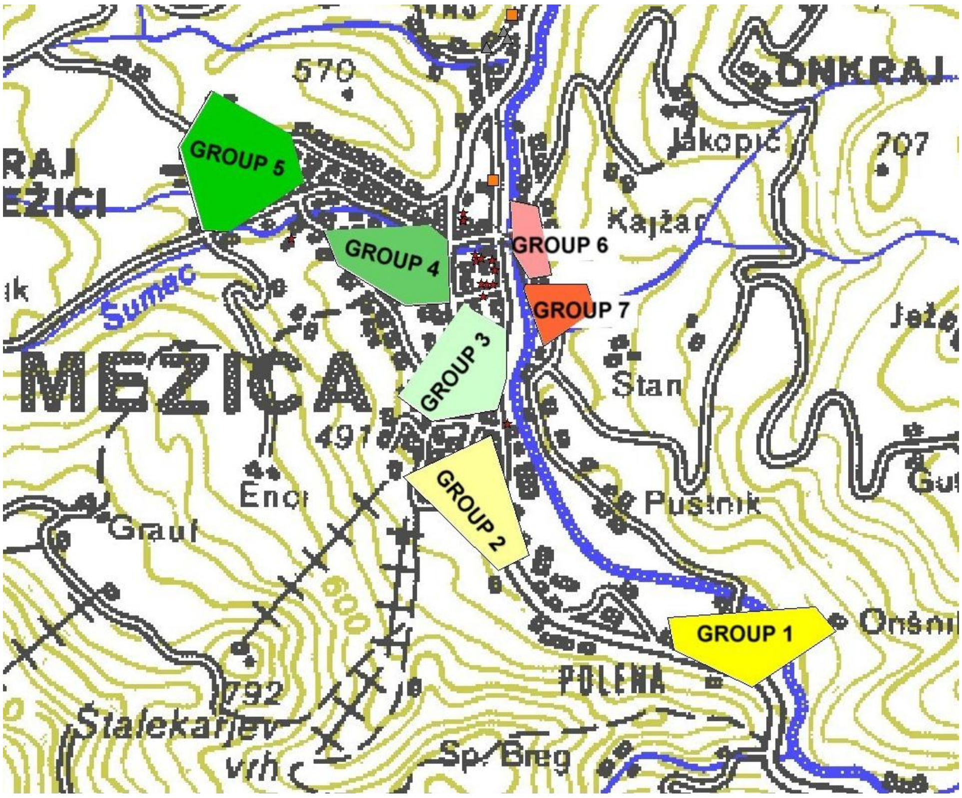


***Figure 11**: Groups locations in the Mežica community*

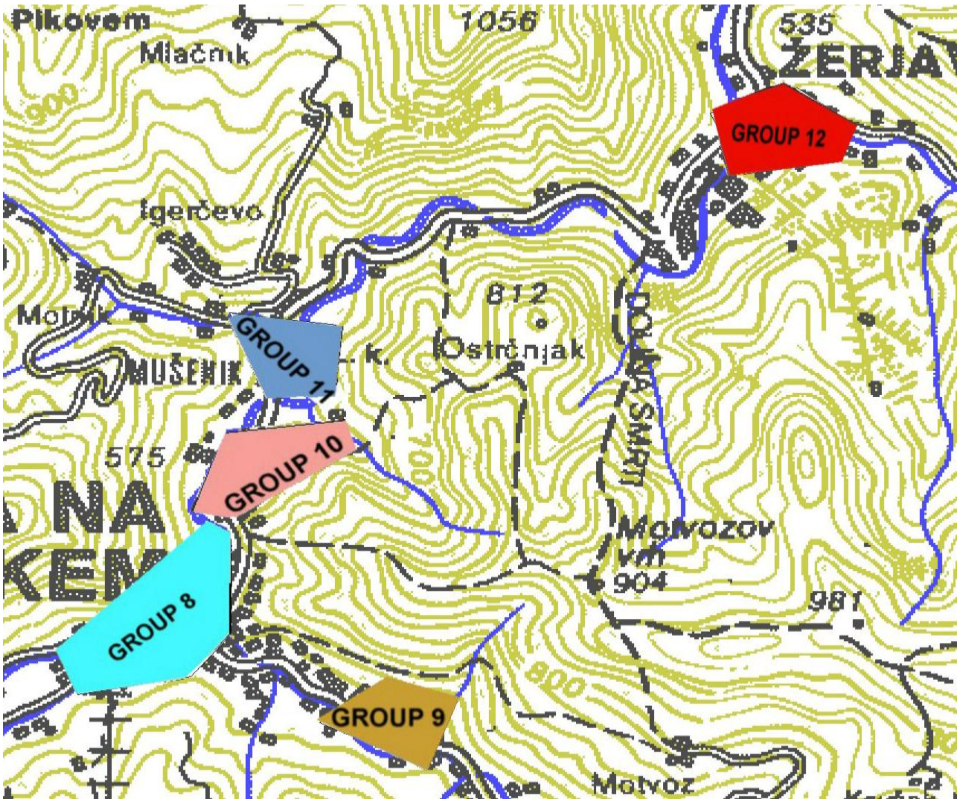


Figure 12: *Groups locations in the Črna na Koroškem community*

In every group, site specific data for soil and dust was used. At the start, a comparison was made of concentrations of lead in soil and dust with blood lead concentrations in children for each group with at least 5 children. The following diagram shows that the group with the highest environmental lead burden also included children with higher blood lead burden (Figure 13).

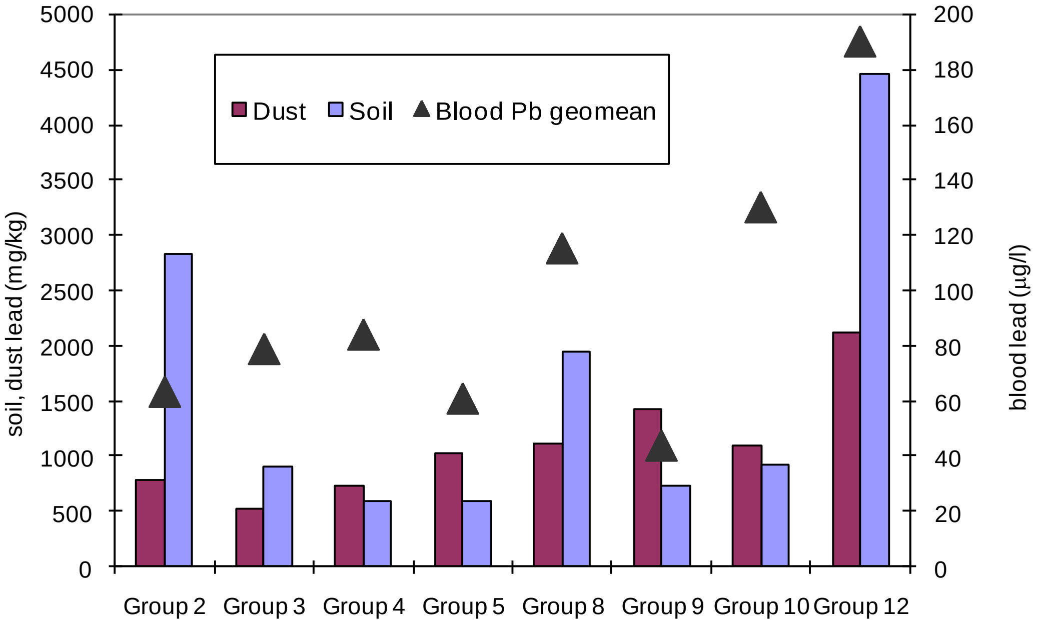


Figure 13: *Dust, soil and blood lead burden for groups with at least 5 children*

Input data and results of calculations for each group are presented in following tables. (Table 14 – 25)

Table 14: *Input data and results of calculations for group 1*

Group 1		
Media	*Mark*	*Concentration*
Dust	*MS2*	*839 mg/kg*
Soil	*M1*	*1410 mg/kg*
Children	*74, 116, 149, 151 (n=4)*	*Range: 33-297 µg/l*
		Geometric mean: 120.5 µg/l
IEUBK predicted geometric mean		*129 µg/l*
IEUBK predicted probability that blood lead concentration will exceed level of concern (≥ 100 µg/l)		*70.7 %*
Proportion of children with blood lead concentration ≥ 100 µg/l		*75 % (3/4)*

Table 15: *Input data and results of calculations for group 2*

Group2		
Media	*Mark*	*Concentration*
Dust	*MS1*	*787 mg/kg*
Soil	*M2*	*2830 mg/kg*
Children	*5, 56, 38, 89, 18, 59, 24, 122, 124, 125, 154, 156 (n=12)*	*Range: 21-167 µg/l*
		Geometric mean: 63.5 µg/l
IEUBK predicted geometric mean		*175 µg/l*
IEUBK predicted probability that blood lead concentration will exceed level of concern (≥ 100 µg/l)		*88.3 %*
Proportion of children with blood lead concentration ≥ 100 µg/l		*16.7 % (2/12)*

***Table 16**: Input data and results of calculations for group 3*

Group3		
Media	Mark	Concentration
Dust	MS3	524 mg/kg
Soil	M3	898 mg/kg
Air	AirM	0.054 $\mu g/m^3$
Children	95, 65, 54, 34, 60, 25, 100, 75, 1, 40, 119, 120, 146, 161, 179, 187, 193 (n=17)	Range: 27-282 µg/l
		Geometric mean: 79.0 µg/l
IEUBK predicted geometric mean		93 µg/l
IEUBK predicted probability that blood lead concentration will exceed level of concern (≥ 100 µg/l)		44.1 %
Proportion of children with blood lead concentration ≥ 100 µg/l		41.2 % (7/17)

***Table 17**: Input data and results of calculations for group 4*

Group4		
Media	Mark	Concentration
Dust	MS5	737 mg/kg
Soil	M5	597 mg/kg
Air	AirM	0.054 $\mu g/m^3$
Children	61, 39, 31, 29, 76, 15, 3, 62, 79, 105, 118, 152, 153, 160, 190 (n=15)	Range: 34-185 µg/l
		Geometric mean: 84.2 µg/l
IEUBK predicted geometric mean		91 µg/l
IEUBK predicted probability that blood lead concentration will exceed level of concern (≥ 100 µg/l)		42.5 %
Proportion of children with blood lead concentration ≥ 100 µg/l		46.7 % (7/15)

***Table 18**: Input data and results of calculations for group 5*

Group5		
Media	Mark	Concentration
Dust	MS6	1024 mg/kg
Soil	M6	593mg/kg
Children	27, 4, 37, 17, 67, 87, 82, 72, 102, 115,121, 123,155, 181, 182, 194 (n=16)	Range: 10-253 µg/l
		Geometric mean: 61.1 µg/l
IEUBK predicted geometric mean		106 µg/l
IEUBK predicted probability that blood lead concentration will exceed level of concern (≥ 100 µg/l)		55.1 %
Proportion of children with blood lead concentration ≥ 100 µg/l		37.5 % (6/16)

Table 19: *Input data and results of calculations for group 6*

Group6		
Media	Mark	Concentration
Dust	MS8	1287 mg/kg
Soil	M4	1030 mg/kg
Children	47, 55 (n=2)	Range: 110-356 μg/l
		Geometric mean: 197.9 μg/l
IEUBK predicted geometric mean		135 μg/l
IEUBK predicted probability that blood lead concentration will exceed level of concern (≥ 100 μg/l)		74.0 %
Proportion of children with blood lead concentration ≥ 100 μg/l		100 % (2/2)

Table 20: *Input data and results of calculations for group 7*

Group7		
Media	Mark	Concentration
Dust	MS4	787 mg/kg
Soil	M4	1030 mg/kg
Children	21 (n=1)	32 μg/l
IEUBK predicted geometric mean		112 μg/l
IEUBK predicted probability that blood lead concentration will exceed level of concern (≥ 100 μg/l)		59.7 %
Proportion of children with blood lead concentration ≥ 100 μg/l		0 % (0/1)

Table 21: *Input data and results of calculations for group 8*

Group8		
Media	Mark	Concentration
Dust	CS2, CS3	1103 mg/kg
Soil	C6	1950 mg/kg
Water	Amerika	8.4 μg/l
Children	68, 66, 81, 91, 70, 42, 90, 83, 128, 133, 141, 145, 171, 175, 177, 178, 186 (n=17)	Range: 28-420 μg/l
		Geometric mean: 115.5 μg/l
IEUBK predicted geometric mean		161 μg/l
IEUBK predicted probability that blood lead concentration will exceed level of concern (≥ 100 μg/l)		84.5 %
Proportion of children with blood lead concentration ≥ 100 μg/l		70.6 % (12/17)

Table 22: *Input data and results of calculations for group 9*

Group9		
Media	Mark	Concentration
Dust	CS1	1426 mg/kg
Soil	C8	734 mg/kg
Air	AirC	0.076 µg/m^3
Children	101, 41, 33, 19, 134, 138, 189 (n=7)	Range: 15-180 µg/l
		Geometric mean: 44.1 µg/l
IEUBK predicted geometric mean		131 µg/l
IEUBK predicted probability that blood lead concentration will exceed level of concern (≥ 100 µg/l)		71.6 %
Proportion of children with blood lead concentration ≥ 100 µg/l		14.3 % (1/7)

Table 23: *Input data and results of calculations for group 10*

Group10		
Media	Mark	Concentration
Dust	CS4	1087 mg/kg
Soil	C5	925 mg/kg
Air	AirR	0.083 µg/m^3
Water	Amerika	8.4 µg/l
Children	23, 12, 30, 96, 6, 97, 13, 8, 98, 78, 48, 112, 129, 130, 137, 142, 163, 173, 183, 191, 192 (n=21)	Range: 42-303 µg/l
		Geometric mean: 130.3 µg/l
IEUBK predicted geometric mean		125 µg/l
IEUBK predicted probability that blood lead concentration will exceed level of concern (≥ 100 µg/l)		68.1 %
Proportion of children with blood lead concentration ≥ 100 µg/l		71.4 % (15/21)

Table 24: *Input data and results of calculations for group 11*

Group11		
Media	Mark	Concentration
Dust	CS5	724 mg/kg
Soil	C4	1890 mg/kg
Water	Amerika	8.4 µg/l
Children	14, 184, 188 (n=3)	Range: 85-180 µg/l
		Geometric mean: 116.4 µg/l
IEUBK predicted geometric mean		144 µg/l
IEUBK predicted probability that blood lead concentration will exceed level of concern (≥ 100 µg/l)		78.0 %
Proportion of children with blood lead concentration ≥ 100 µg/l		66.7 % (2/3)

***Table 25**: Input data and results of calculations for group 12*

Group12		
Media	*Mark*	*Concentration*
Dust	*ZS2*	*2126 mg/kg*
Soil	*C1*	*4470 mg/kg*
Air	*AirZ*	*0.072* $\mu g/m^3$
Children	*99, 11, 45, 10, 52, 110, 135, 144, 147, 168,170 (n=11)*	*Range: 58-500 μg/l*
		Geometric mean: 190.3 μg/l
IEUBK predicted geometric mean		*258,7 μg/l*
IEUBK predicted probability that blood lead concentration will exceed level of concern (≥ 100 μg/l)		*97.8 %*
Proportion of children with blood lead concentration ≥ 100 μg/l		*90.9 % (10/11)*

Following figures represent comparison between observed and IEUBK model predicted blood lead concentrations and proportions of children with elevated blood lead levels (Figure 14 – 17).

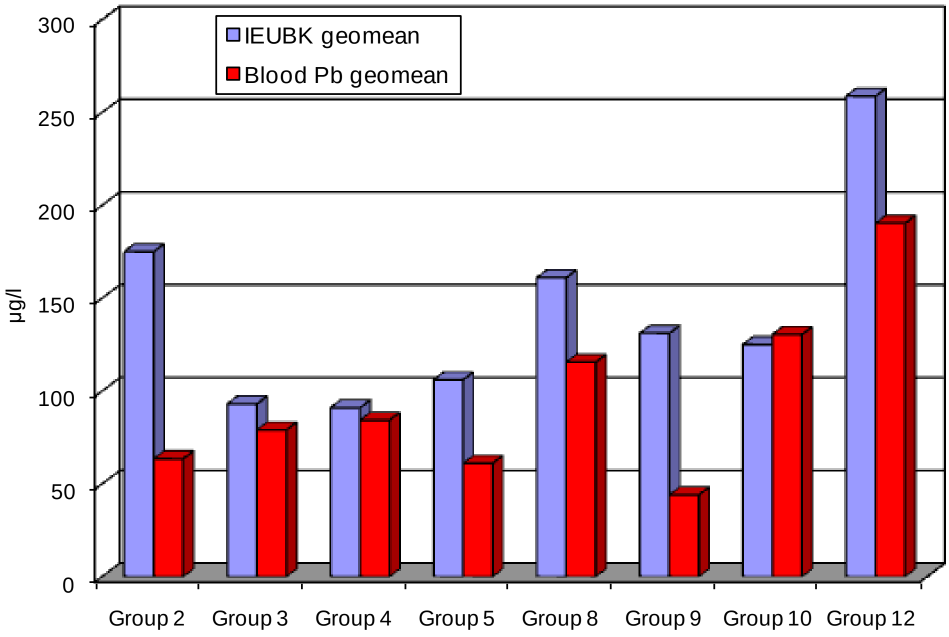


***Figure 14**: Comparison between the IEUBK predicted and observed blood lead geometric mean concentrations by group (for groups including at least 5 children)*

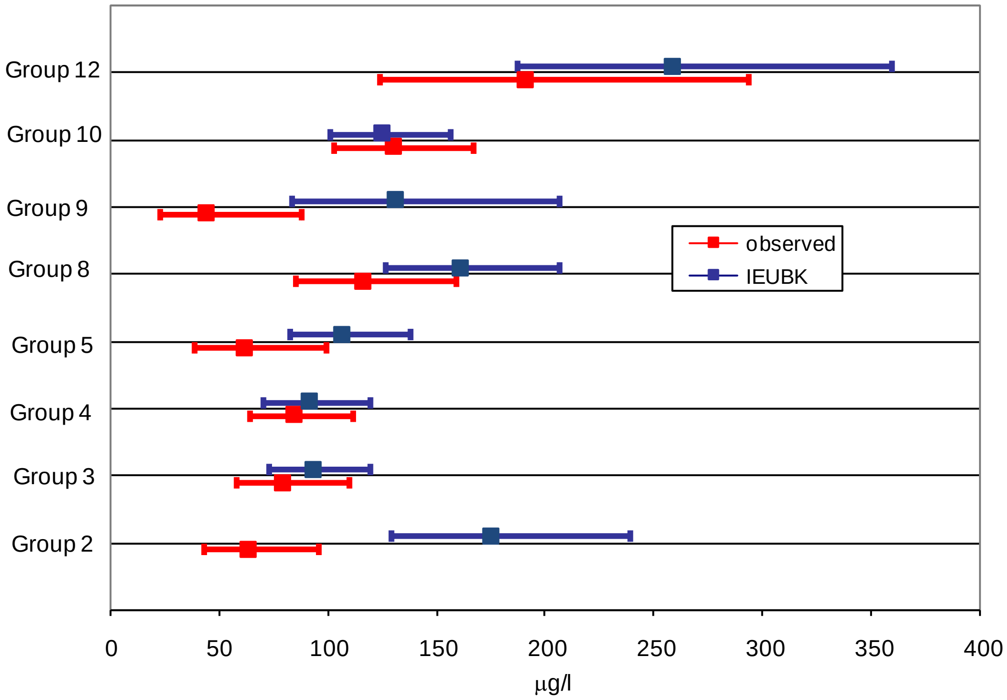


Figure 15: *Comparison of 95% confidence intervals of observed and IEUBK model predicted blood lead geometric mean concentrations by group, (for groups including at least 5 children)*

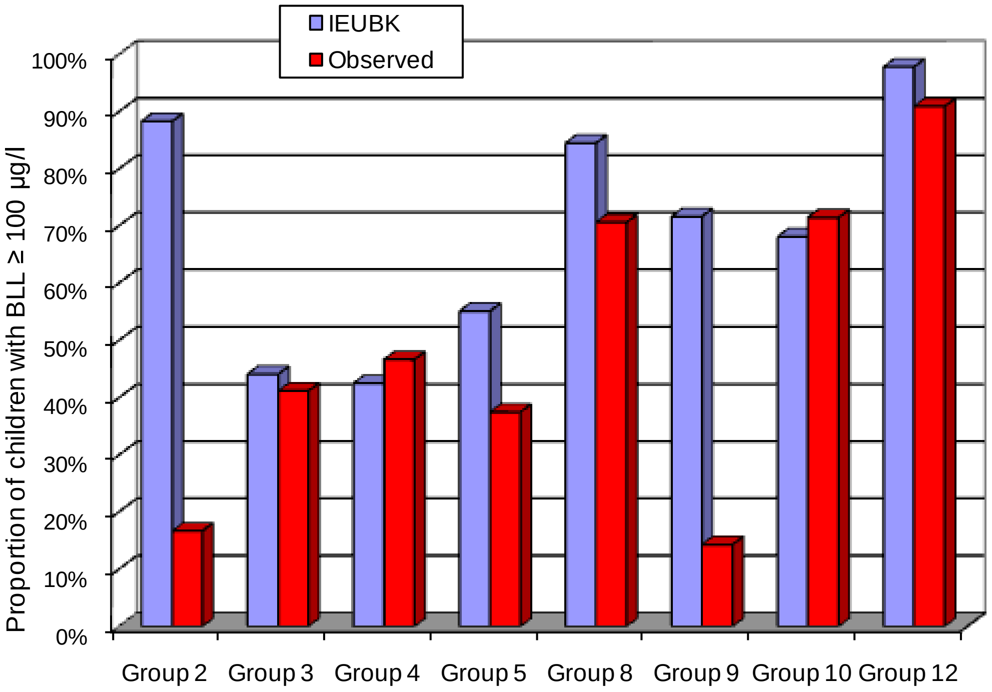


Figure 16: *Comparison of the IEUBK predicted and observed proportion of children with elevated (≥ 100 µg/l) blood lead levels by group (for groups including at least 5 children)*

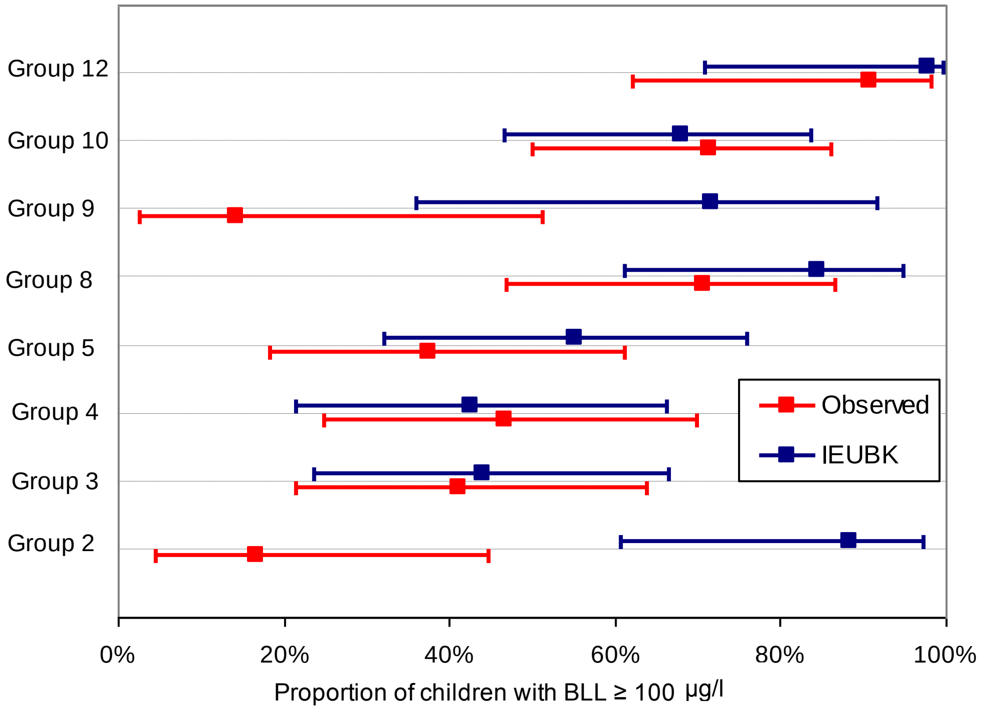


Figure 17: *Comparison of 95% confidence intervals of observed and IEUBK model predicted proportion of children with elevated (≥ 100 µg/l) blood lead levels by group (for groups including at least 5 children)*

The IEUBK predicted values were also compared to observed values for all groups together (Table 26).

Table 26: *Results of calculations for whole area (all groups) under study*

IEUBK geometric mean predictions average	*138.1 µg/l*
Geometric mean (children in groups)	*93.0µg/l*
Geometric mean (all children in study)	*95.8 µg/l*
Probability that blood lead concentration in child will exceed level of concern (≥100 µg/l), (IEUBK prediction)	*67.9 %*
Proportion of children with blood lead concentration ≥ 100 µg/l (children in groups)	*53.2 % (67/126)*
Proportion of children with blood lead concentration ≥ 100 µg/l (all children in study)	*54.6 % (107/196)*

The differences between predicted and observed values were not statistically significant. (Table 27)

***Table 27*:** *McNemar's test for whole area (all groups) under study*

		Observed ≥100 μg/l	
		Yes = 1	*No = 0*
Predicted ≥ 100 μg/l	*Yes = 1*	39	*27*
	No = 0	38	*22*
		$\chi^2=1,54$ *p=0,21*	

4.5 Comparison of the IEUBK model predictions with empirical data (locations)

Another approach was also tested where we simply created a net of 1000 by 1000 meters squares over the map of the entire area of the study. Each square represented one group in which children living in that square, and the sampling points for environment media located in that square were classified. Each group was named according to a letter and number marking the lines forming the square. (Figure 18) Predicted blood lead geometric mean and probability that the chosen blood lead level of concern (≥100 μg/l) will be exceeded were compared with geometric mean and the proportion of children with blood lead level ≥ 100 μg/l calculated from the observed data. Only squares including at least one sampling point for soil and house dust lead and at least 5 children were considered in the study. Since there were different sampling points for the same environmental factors in some groups, calculations using the average values for those factors were performed. Since each group must include data on environmental lead and blood lead, only 7 groups were formed. To avoid confusion in further text, a term "locations" is being used for these groups.

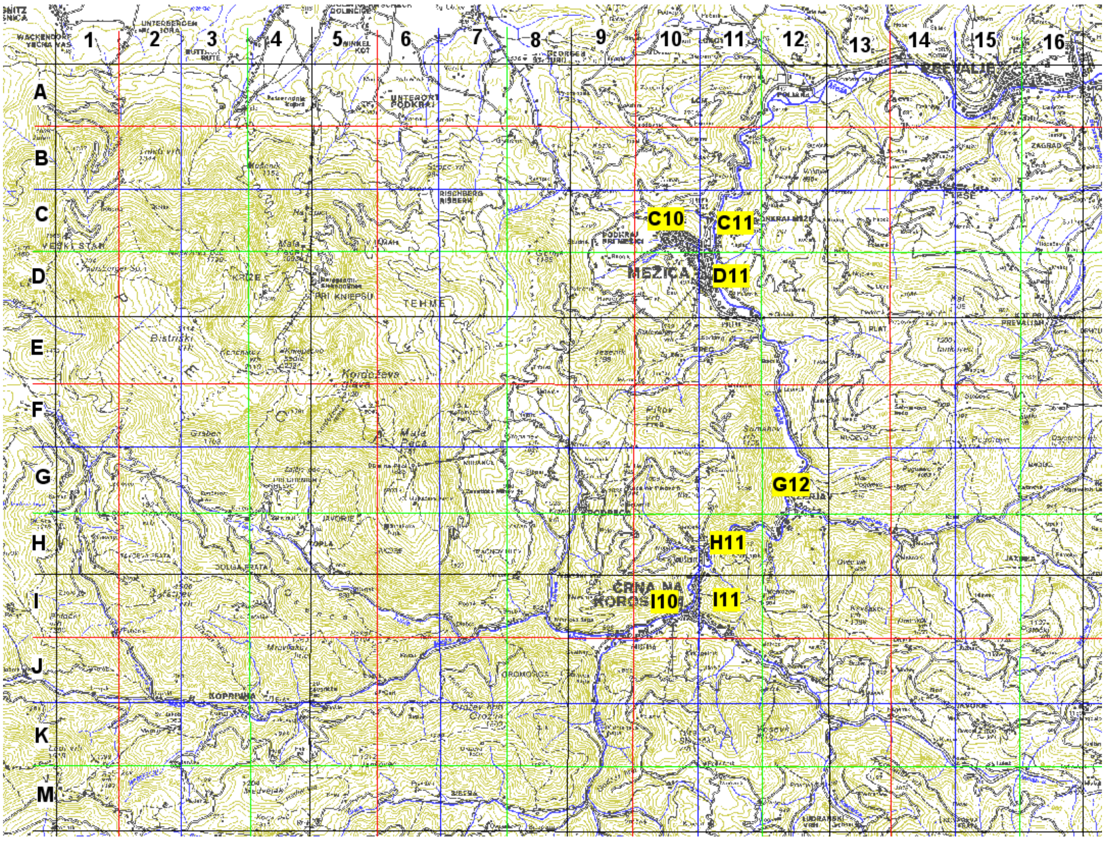


Figure 18: *Locations of groups defined by square net 1000 by 1000 meters*

We started with the diagram presenting concentration of lead in soil, house dust and blood of children for each group. In this diagram, average concentrations for lead in environmental media were used. (Figure 19)

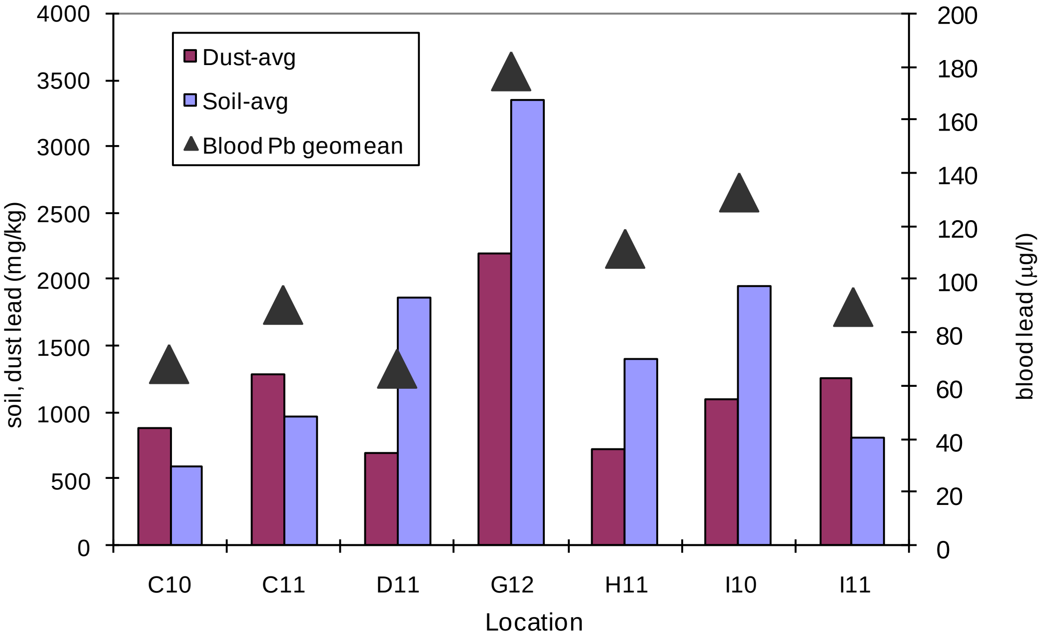


Figure 19: *Dust, soil and blood lead burden for locations with at least 5 children*

The diagram shows that children living on locations with higher lead concentrations in yard soil and house dust have higher blood lead concentrations.

Input data and calculation results for each location are presented in the following tables (Table 28 – 34)

Table 28: *Input data and results of calculations for location C10*

Location C10		
Parameter	*Mark*	*Value*
Soil	*M5, M6*	*595 mg/kg (597, 593)*
Dust	*MS5, MS6*	*880.5 mg/kg (737, 1024)*
Children	*3,4,15,17,20,27,37,67,72,76,79,82,87,102,115, 121,123,152,153,155,181,182,194 (n=23)*	*Range: 10 to 253 μg/l*
		Geo. mean: 68.1μg/l
IEUBK predicted geometric mean calculated from average values of lead concentrations for environmental factors		*99 μg/l*
Proportion of children with blood lead concentration ≥ 100 μg/l		*39.1 % (9/23)*
IEUBK predicted probability that blood lead concentration will exceed level of concern (≥ 100 μg/l) – using average values of lead concentrations for environmental factor		*49.2 %*

Table 29: *Input data and results of calculations for location C11*

Location C11		
Parameter	*Mark*	*Value*
Soil	*M4, M7, M8*	*971 mg/kg (1030, 947, 936)*
Dust	*MS8*	*1287 mg/kg*
Children	*26,28,32,43,46,47,49,50,55,62,105,107,113,114, 118,150,180 (n=17)*	*Range: 25 to 356 μg/l*
		Geo. mean: 90,4 μg/l
IEUBK predicted geometric mean calculated from average values of lead concentrations for environmental factors		*133 μg/l*
Proportion of children with blood lead concentration ≥ 100 μg/l		*47.1 % (8/17)*
IEUBK predicted probability that blood lead concentration will exceed level of concern (≥ 100 μg/l) – using average values of lead concentrations for environmental factor		*72.9 %*

Table 30: *Input data and results of calculations for location D11*

Location D11		
Parameter	*Mark*	*Value*
Soil	*M2, M3*	*1864 mg/kg (2830, 898)*
Dust	*MS1, MS3, MS4*	*699,3 mg/kg (787, 524, 787)*
Air	*AirM*	*0,054 μg/m*^{3}
Children	*1,16,18,21,24,29,31,34,39,40,54,56,59,60,65, 75,89,95,100,117,119,120,122,124,125,127, 146,148, 154,156,159,160,161,179, 187, 189,193 (n=37)*	*Range: 21 to 282 μg/l*
		Geo. mean: 66.4μg/l
IEUBK calculations using average values of lead concentrations for environmental factors		*139 μg/l*
Proportion of children with blood lead concentration ≥ 100 μg/l		*24.3 % (9/37)*
IEUBK predicted probability that blood lead concentration will exceed level of concern (≥ 100 μg/l) – using average values of lead concentrations for environmental factor		*76.1 %*

Table 31: *Input data and results of calculations for location G12*

Location G12		
Parameter	*Mark*	*Value*
Soil	*Č1*	*3350 mg/kg*
Dust	*ŽS1, ŽS2*	*2201,5 mg/kg (2277, 2126)*
Air	*AirZ*	*0,072 μg/m*^{3}
Children	*10,11,45,52,99,110,144,147,170 (n=9)*	*Range: 58 to 364 μg/l*
		Geo. mean: 178,3μg/l
IEUBK calculations using average values of lead concentrations for environmental factors		*235 μg/l*
Proportion of children with blood lead concentration ≥ 100 μg/l		*88.9 % (8/9)*
IEUBK predicted probability that blood lead concentration will exceed level of concern (≥ 100 μg/l) – using average values of lead concentrations for environmental factor		*96.6 %*

Table 32: *Input data and results of calculations for location H11*

Location H11		
Parameter	*Mark*	*Value*
Soil	*Č4, Č5*	*1407,5 mg/kg (1890, 925)*
Dust	*ČS5*	*724 mg/kg*
Children	*8,13,14,48,78,98,130,163,183,184,188 (n=11)*	*Range: 62 to 303 μg/l*
		Geo. mean: 111,5 μg/l
IEUBK calculations using average values of lead concentrations for environmental factors		*124 μg/l*
Proportion of children with blood lead concentration ≥ 100 μg/l		*54.6 % (6/11)*
IEUBK predicted probability that blood lead concentration will exceed level of concern (≥ 100 μg/l) – using average values of lead concentrations for environmental factor		*67.6 %*

***Table 33**: Input data and results of calculations for location I10*

Location I10		
Parameter	Mark	Value
Soil	Č6	1950 mg/kg
Dust	ČS2, ČS3	1103 mg/kg (1092, 1114)
Water	Amerika	8,4 mg/l
Children	23,35,42,44,57,66,68,70,77,81,83,90,91, 104,128,133,137,141,142,143,145,162, 169, 1,173,175,177,178,186,191,192 (n=31)	Range: 28 to 480 μg/l
		Average: 162,3 μg/l
		Geo. mean: 129,2μg/l
IEUBK calculations using average values of lead concentrations for environmental factors		161 μg/l
Proportion of children with blood lead concentration ≥ 100 μg/l		71.0 % (22/31)
IEUBK predicted probability that blood lead concentration will exceed level of concern (≥ 100 μg/l) – using average values of lead concentrations for environmental factor		84.5 %

***Table 34**: Input data and results of calculations for location I11*

Location I11		
Parameter	Mark	Value
Soil	Č7, Č8	815,5 mg/kg (897, 734)
Dust	ČS1, ČS4	1256,5 mg/kg (1426, 1087)
Air	AirC, AirR	0,0795 μg/m^3 (0,076, 0,083)
Children	6,12,19,30,33,41,88,96,97,101,112,129,134,138,139 (n=15)	Range: 15 to 299 μg/l
		Geometric mean:89,6μg/l
IEUBK calculations using average values of lead concentrations for environmental factors		126 μg/l
Proportion of children with blood lead concentration ≥ 100 μg/l		60 % (9/15)
IEUBK predicted probability that blood lead concentration will exceed level of concern (≥ 100 μg/l) – using average values of lead concentrations for environmental factor		68.9 %

The following figures represent a comparison between the observed and IEUBK model predicting blood lead concentrations and proportions of children with elevated blood lead levels (Figure 20 – 23).

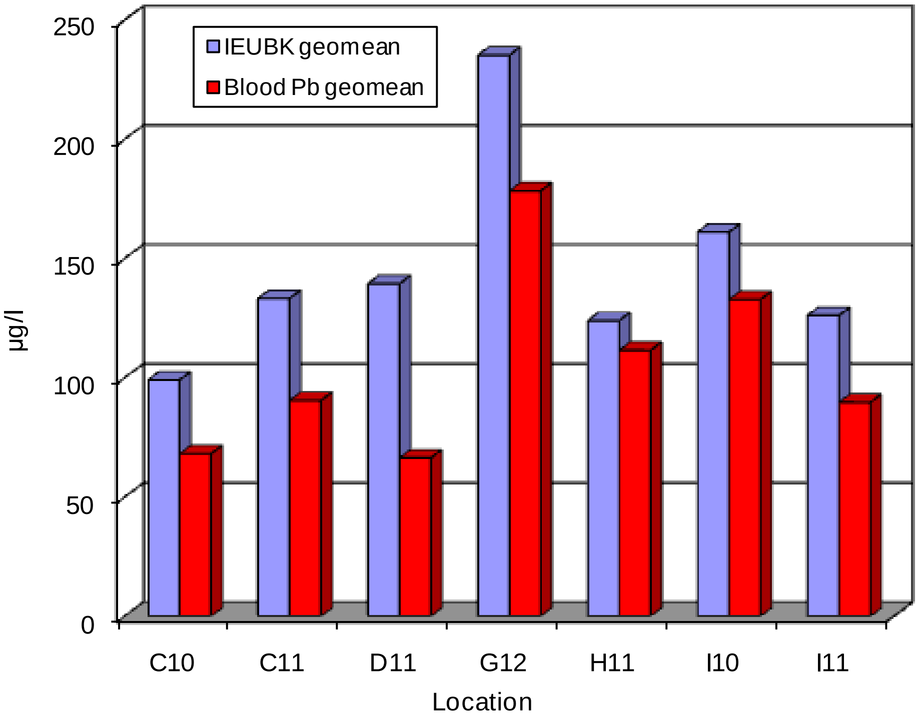


Figure 20: *Comparison between the IEUBK predicted and observed blood lead geometric mean concentrations by location*

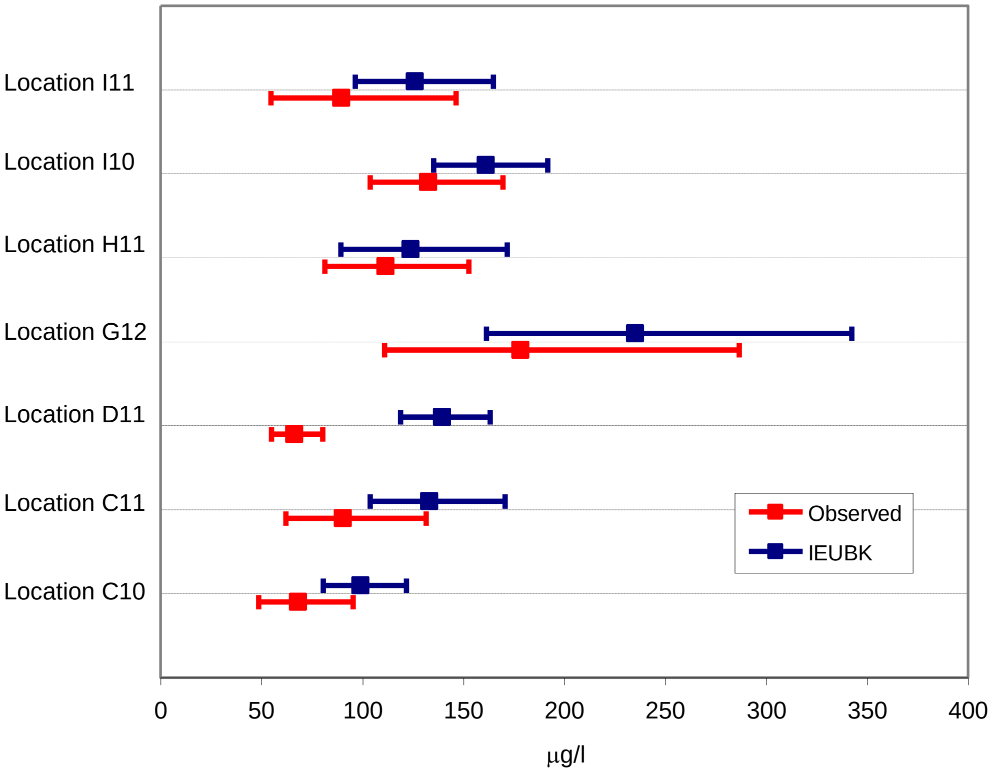


Figure 21: *Comparison of 95% confidence intervals of observed and IEUBK model predicted blood lead geometric mean concentrations by location*

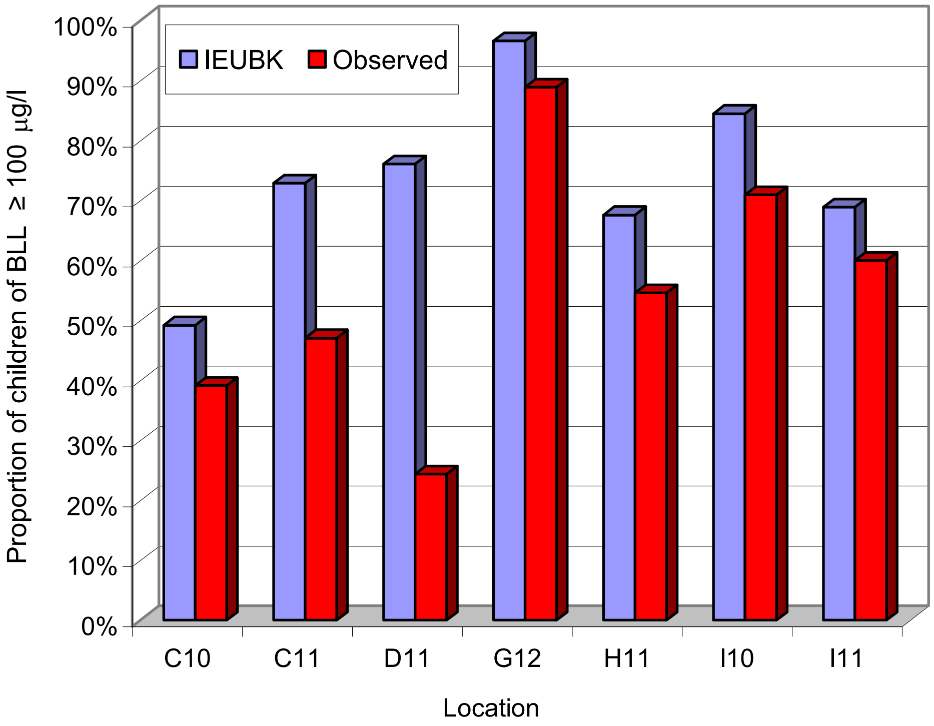


***Figure 22**: Comparison of the IEUBK predicted and observed proportion of children with elevated (≥ 100 µg/l) blood lead levels by location*

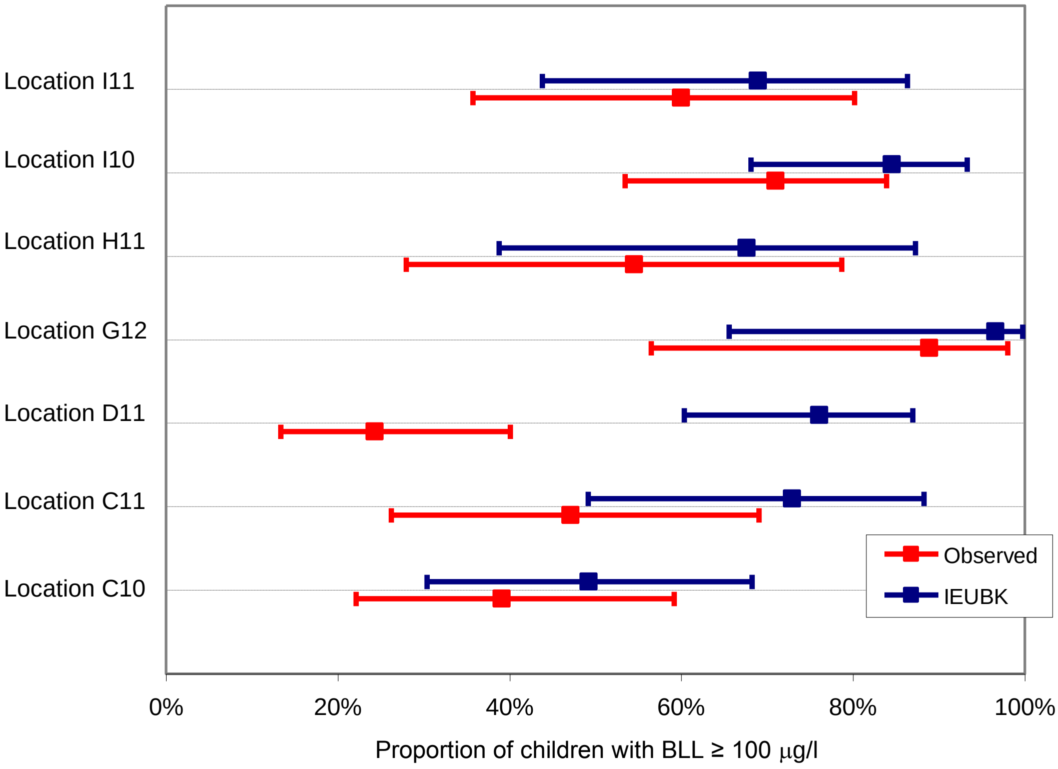


***Figure 23**: Comparison of 95% confidence intervals of observed and IEUBK model predicted proportion of children with elevated blood lead levels by location*

The IEUBK predicted values were also compared to observed values for all locations together (Table 35).

Table 35**:** *Results of calculations for all location included in study*

Proportion of children with elevated blood lead concentration (≥ 100 μg/l)	*49.7 % (71/143)*
Average IEUBK predicted probability that blood lead concentration in child will be elevated (≥ 100 μg/l), calculated from predictions for locations	*73.1 %*

The differences between predicted and observed values were statistically significant (Table 36).

Table 36**:** *McNemar's test for whole area (all locations) under study*

		Observed ≥100 μg/l	
		Yes = 1	No = 0
Predicted ≥ 100 μg/l	Yes = 1	60	11
	No = 0	59	12
		χ^2=31,56 P<0,01	

5 DISCUSSION

5.1 Study

The Meža Valley has been known in Slovenia as an important industrial area especially due to large ironworks Železarna Ravne and the Lead and Zinc mine in Mežica. The mining tradition, lasting for five centuries, was typical for the Upper Meža Valley. In that part of the valley, there is a population of almost 7500 people. Three towns: Črna na Koroškem, Mežica and Žerjav are important. Mežica had had a lead and zinc mine which was in operation until 1989, while the process of lead batteries production still continues in Žerjav. The primary raw material, lead sulphide ore, was replaced by the secondary raw material – old lead batteries.

All that industry also had several negative side effects. Nowadays, with the problems of occupational exposure mainly resolved, exposure to environmental pollution and the consecutive influences on human health are the main cause for professional concern.

In addition to 500 years of mining tradition, there have also been 50 years of relevant health studies. Virtually all studies have shown large lead concentrations in environment factors and large lead burden in the Upper Meža Valley inhabitants. The Comparative Study of the Environment Pollution in the Upper Meža Valley between 1989 and 2001 is the last comprehensive study of lead and other heavy metals burden in the Upper Meža Valley in which environment pollution and large burden in men were confirmed again. That last study also triggered the start of the project "Living with Lead" which is being carried out by the Regional Institute of Public Health Ravne na Koroškem. A part of the project also involves the testing of blood samples of 3 year-old children. Detected concentrations are still well above 100 μg/l and should be lowered within foreseeable time.

The final goal of our research is to lower lead blood burden under the level of concern. To achieve it, human exposure and lead intake of lead have to be reduced. There are many different strategies to do that and it needs to be decided which strategies are the most efficient.

Different studies[80,81] showed that the IEUBK model is a tool which can be helpful in identification of the most important factors of children exposure to lead. The IEUBK model also allows calculating lead levels in these factors, which should be achieved if the children blood burden wants to be lowered. If there is a very clear goal set for children blood burden (95% under 100 μg/l), the best paths to achieve it are still under discussion. It is our goal to clear them up. It was decided to test the IEUBK model with data on environment lead concentrations for the Upper Meža Valley and then to compare the results with the actual data on children blood lead burden. The calculations with the model should reveal if measured blood lead concentrations in children reflect environmental pollution level and indicate the most important exposure factors. Environmental remedial measures should address these factors and in this respect, the IEUBK model could be helpful in deciding on priorities of the Upper Meža Valley environmental remedial activities.

5.2 Material and methods

5.2.1 The IEUBK model

The IEUBK Model for Lead in Children was used without modifications, with available site specific data for the Upper Meža Valley environment. The model was evaluated by different studies and has been considered as a sufficiently reliable tool for use in our study. It was our main interest to use the model as a tool, to gain additional information about exposure factors to lead for children from the Upper Meža Valley. These pieces of information should help us in planning remedial strategies.

5.2.1.1 Evolution of the model

In 1985, the EPA Office of Air Quality Planning and Standards (OAQPS) initiated a project that would allow the calculation of blood lead concentrations in children exposed to differing arrays of concentrations of lead in air, soil, and dust. This model, called the Uptake/Biokinetic (or UBK) model for lead, was a computer simulation model based on the biokinetic model for lead in children developed by N. Harley and T. Kneip in 1985.[82] The biokinetic parameters for the UBK model were extrapolated from long-term feeding studies on infant and juvenile baboons[83] autopsy data on human children, human infant feeding studies, and other sources. Study of lead retaintion and tissue distribution in infant and adult monkeys showed reasonable agreement with the data about lead absorbtion and tissue distribution in humans, meaning that the infant monkeys represent an adequate model for studies of lead biokinetics in children.[28] The exposure model that was coupled to the biokinetic model was developed by EPAs Office of Air Quality Planning and Standards. Model calibration and validation was done using data from the 1983 EPA/CDC/Montana study on children in East Helena, Montana, who lived in the vicinity of a large primary lead smelter.[84] The modeling approach was reviewed and approved by EPA's Clean Air Science Advisory Committee in 1990.

5.2.1.2 Using the IEUBK model for risk estimation

The IEUBK Model for lead is designed to facilitate:

- rapid delineation of the relationship between environmental lead and blood lead in children; and
- calculation of the risk of elevated blood lead (i.e., the probability of a given child or a group of children having blood lead concentrations exceeding a specified level of concern).

As such, the IEUBK Model provides a tool for site-specific risk assessment for young children exposed to lead from different media and through different pathways in their environment, with particular emphasis on lead in air, water, soil, and household dust. Many other applications are possible. The intended applications of the IEUBK model are to:

- Provide a summary of children's long-term, primarily residential, exposure to lead.
- Provide a best estimate of the geometric mean blood lead concentration for a typical child aged 6 to 84 months, assumed to reside at a given residence.
- Provide a basis for estimating the risk of elevated blood lead (i.e., for exceeding a designated blood lead concentration of concern) for a hypothetical child of specified age with given site-specific residential lead exposure.
- Provide a basis for estimating the risk of elevated blood lead concentrations among early pediatric populations in a given neighborhood by aggregating the individual residential risk estimates.

- Predict likely changes in risk of elevated blood lead concentrations from exposure to soil, dust, water, or air lead following abatement actions designed to reduce exposure levels from one or more environmental media.
- Provide assistance in determining appropriate soil or dust lead target cleanup levels at specific residential sites.
- Provide assistance in estimating blood lead concentrations associated with soil or dust lead concentrations at undeveloped residential sites that may be developed in the future.

The risk estimates are calculated for a hypothetical child or a hypothetical population of children who could be occupying the specific household at the time of the measurements or at some future time. A site-specific risk assessment requires site-specific soil and dust concentrations, and some of the absorption parameters may depend on specific characteristics of the soil and dust at the site.[66]

5.2.1.3 Validation of the model

EPA's experts say that IEUBK model is valid, because following conditions are satisfied:

- The model is biologically and physically plausible and incorporates the best available empirical data on parameters.
- Numerically accurate algorithms are used and the accuracy of the computer codes for these algorithms has been verified;
- The model provides some satisfactory empirical comparisons of model output with real-world data.

They believe that the scientific basis and computational correctness of the IEUBK model is sound, and that the IEUBK model provides valid prediction of observed blood lead concentrations from representative populations of children with typical exposure. The empirical comparisons in which there are differences between observed and predicted blood lead concentrations underscore the importance of valid exposure scenarios as input. They also show the importance of valid blood lead data from truly representative population sampling methods when interpreting these empirical comparisons.[66]

In one evaluation of the model author points out that the model is not empirical and that the data required for validation is much more extensive than the overall input/output data, if the model is to be used in a predictive model where a previous set of parameter conditions has not been experienced.[85]

5.2.1.4 Limitations of the model

The IEUBK Model is designed to evaluate relatively stable exposure situations, rather than rapidly varying exposures. The model does not report each iterative calculation; rather, it reports one-year average blood lead concentrations. Because the IEUBK Model allows changes in exposure to environmental lead concentrations only at one year intervals, and provides output at only one year age intervals, changes in exposure are smoothed over one year. The model cannot be used to predict the effects of short term exposure episodes, such as exposure over a few days or weeks to lead dust and airborne particles.[66]

5.2.1.5 Empirical comparisons of the model

Comparison of the IEUBK model output with empirical human blood lead data has two requirements.

- The first one is that the child's total lead exposure is completely and accurately characterized by the empirical data, including site-specific data on environmental lead concentration, media ingestion, and bioavailability.
- The second requirement is that the blood lead data from the field study are accurate and typical for that exposure scenario.

A typical child may not have the exposure described by the measured and default parameters of the model, or a child may also respond atypically to the measured and default parameters. The solution is to find the correct set of parameters (measured or site-specific alternatives to default) that describes the child's site-specific exposure or response to exposure.

Analyses of several data sets so far indicate that the model satisfactorily predicts blood lead concentrations[86,87,88] for the overall sample populations in specific neighborhoods. Further analyses will be needed to determine if empirical comparisons are as strong for subpopulations defined by factors such as differences in age, differences in contact or behavior that affected the amount of soil ingested, suspected or possible differences in bioavailability, differences in contribution of soil to household dust, and identifiable biases in recruitment of children.[66]

5.3 IEUBK input and blood lead data

The study used data on environmental exposure from the Comparative Study of the Environment Pollution in the Upper Meža Valley between 1989 and 2001. The data on lead in drinking water has been obtained from the Regional Institute of Public Health Ravne na Koroškem archives.

5.3.1 The Comparative Study of the Environment Pollution in the Upper Meža Valley between 1989 and 2001

The last comprehensive study of lead and other heavy metals burden in the Upper Meža Valley addressing different areas from the environment to food, animals and people is The Comparative Study of the Environment Pollution in the Upper Meža Valley between 1989 and 2001. The study was carried out by the ERICo Institute in the period 2001 – 2002. The study results showed that heavy metals concentrations and especially lead are still high and that environment remediation should be carried out. Lead concentrations were higher than those in a control area or control data for almost all segments of the study, but we were mainly interested in the data which are closely connected to human lead burden and could be used in the IEUBK model.

5.3.1.1 Influences on human health

The goal of the study was to estimate lead intake from many different sources and lead blood burden was chosen as an appropriate indicator. Two study groups were formed. The first one included inhabitants of the Upper Meža Valley and the second one inhabitants of the Lower Meža Valley. For each group, three subgroups were formed. Children under 3 years of age were in the first subgroup, children between 11 and 12 years of age were in the second and army recruits between 18 and 26 years of age were represented in the third subgroup. The highest lead concentration of 146.3 μg/l has been observed in 3 year-old children living in the Upper Meža Valley.

Also in the other two groups, blood lead level was higher for the residents of the Upper Meža Valley than for those living in the Lower Meža Valley.[46]

5.3.1.2 Lead in air (dust)

The focus of the study was on respirable (diameter < 2.5μm) and inhalable (2.5 – 15 μm) particles in air and heavy metals concentrations (Pb, Cd, As, Zn, Hg) in them. The measurement was carried out on 4 locations in the Upper Meža Valley and a control location. The results shoved that there were no important differences in the concentration of particles in the air; on the other hand concentrations of metals, lead in particular, were much higher in the Upper Meža Valley than in the control location.

The chapter on air quality also included study of dust. House dust and road dust samples were collected and analyzed on heavy metals presence. House dust is more important for the evaluation of human exposure. It was collected from vacuum cleaner bags. One sample was made up from three sub samples from three households in the same neighborhood. The samples were collected on 15 locations. When the results are compared with worldwide average lead concentrations in residential dust (Table 37), it can be concluded that lead concentrations measured in 2002 in the Upper Meža Valley are higher for all 15 sampling locations. Average concentration is 2.2 times higher and maximum concentration is 4.5 times higher than worldwide average.[89] One interesting observation is that worldwide average concentrations from 1979 were used, i.e. values from the time when leaded gasoline was still in use. Another important fact is that residential dust consists of house and attic dust and that measured lead concentrations in attic dust were even higher than in house dust.[90] Average lead concentration in house dust was 2.6 times lower than in 1989.[46]

***Table 37:** Worldwide average values for lead in uncontaminated soil and residental dust (Clarke concentrations, Bowen 1979)*[89]

Heavy metal	*value*	*Uncontaminated soil [g/t]*	*Road dust [g/t]*	*Residential dust [g/t]*
Lead	*average*	*35*	*2000*	*500*
	range	*2-300*	*500-4000*	*300-1000*

5.3.1.3 Lead in soil

This chapter of the study deals with the contamination of garden soil, garden vegetables, feeding stuff, forest soil, mushrooms and wild berries. The analyses on concentrations of Pb, Cd, Hg, As and Zn were carried out and the results were evaluated. Elevated Pb concentrations were confirmed.

Since our focus was on lead and the IEUBK model, only the results for lead contamination of garden soil are presented here. Sampling points for garden soil were located in populated areas, which was not the case for forest soil. Data on lead contamination of home grown vegetables could also play an important role in the IEUBK model, but better data on consumption patterns would be needed to make it useful.

20 samples of garden soil were taken on different locations. The analyses were carried out and the results were compared with defined imission threshold values (Table 38). In 19 out of 20 samples, lead concentration exceeded a critical threshold value. Average lead concentration in garden soil was 1412.6 mg/kg with a range from 511 mg/kg to 4470 mg/kg. The maximum value was 8.4 times higher than a critical imission threshold value and over 100 times higher than lead concentration in a soil sample from the control area.[46]

Table 38: *Immission lead threshold values in soil (Decree on the Limit, Warning and Critical Concentration Values of Dangerous Substances in Soil, Of.J. RS No. 68/96)* [91]

Threshold values	*Pb (mg/kg)*
Boundary immission value	*85*
Warning immission value	*100*
Critical immission value	*530*

5.3.2 Blood lead data

The Comparative Study of the Environment Pollution in the Upper Meža Valley also triggered the start of the project “Living with Lead” carried out by the Regional Institute of Public Health Ravne na Koroškem. The project focuses on young children which are the group with the highest health risk due to lead exposure. A part of the project also tests blood samples of 3-year old children. In the period from 2004 to 2007, 184 children were included, 179 of these came from the Upper Meža Valley area. The results show that lead blood levels in many children are still well over 100 μg/l, marking the action level for the Center for Disease Control and Prevention (CDC) in case of children under 7 years of age. So far, the results have shown the lead blood level equal or higher than 100 μg/l in 96 of 179 tested children. Blood burden data from both studies were merged for the purpose of our study. The same methodology carried out by the same laboratory was used in both studies.

The data used in our study was not collected for the purpose of the study, resulting in limitations in the methodology and conclusions. The data on environmental lead pollution was a couple of years older than data on blood lead burden. Since the blood lead concentration only reflects exposure for the last two months, it was questionable whether the environmental data could describe exposure sufficiently. However, the IEUBK model can also be used to predict blood lead levels of future inhabitants of undeveloped residential sites. In addition, a soil lead level should not change much in the undisturbed environment and according to the model; the indoor dust lead concentration is mostly the function of soil lead concentration, so again, dust lead concentrations should not change a great deal over time. The model predictions would probably agree better with observed blood lead levels if blood lead samples were collected only few months after environmental samples, but even so differences should not be too large. Some of the environmental data reflects a short-term period situation, yet long term averages would be better for modeling purpose. Blood lead concentration of each child reflects the exposure for last two months prior to sampling, while the model predicts annual averages. Since the blood samples were collected during the entire year and for a period of four years, this difference is of lesser importance for mean blood lead concentrations of groups of children compared to predicted values. Considering all that, the model could be used in a way it was employed in our study.

Sampling and laboratory procedures for data gathering in the study were carried out by well established laboratories with professional personnel and equipment. Methods and equipment used were validated and proper quality control was assured. Therefore, measurement uncertainty should not play the most important role when interpreting the results. The lack of site specific data enabling a sufficient description of a selected area of concern was much bigger problem in our case. Sampling points for measuring of lead concentrations in environmental factors were rather scattered around the whole area, thus only a small area, including a small number of tested children, could be sufficiently described with each set of data. Because of the small sample size, it was difficult to draw strong conclusions as the IEUBK predicted values were compared to empirical values.

Additional collection and use of site-specific information, particularly site-specific bioavailability and ingestion rates, would have improved the application of the model. There should be more samples of soil, house dust, air and drinking water taken on more locations. Site specific bioavailability of soil and dust lead should be determined. Shorter time between environmental and blood lead data collection would also mark an improvement. Exposure of children should be better described by gathering data on exposure from diet and some other environmental factors (paint, home remedies). Data on behavior patterns and daily movement of children would also improve information about exposure of children. On the other hand, more blood samples of each child during the year would improve observed blood lead data. We understand that the IEUBK model needs very specific data input – lead concentrations for many different exposure factors for specific house in the best case scenario.

There are some other interesting points which might be considered when interpreting the results. The first one is bioavailability. It is suggested that bioavailability of lead in the former mining area, where most lead is found in form of sulfide, is lower than default value in the IEUBK model.[92] This could partially explain why blood lead values predicted by the IEUBK model were higher than empirical ones. Another factor is the difference in behavior patterns between inhabitants of the Upper Meža Valley and typical population used for estimating default values of the model. As we already mentioned, further studies would be needed to come up with conclusions about these factors.

5.4 Results

5.4.1 Blood lead levels according to sex and community of residence

When comparing blood lead levels according to sex, there were larger proportions of boys and children from the Črna na Koroškem community with elevated blood lead levels. Observed differences were confirmed as statistically significant by a chi-square test. Higher blood lead burden in the boys has also been observed in some other studies, but differences were not evaluated as important and were not discussed further.[93,94] In one study of lead content in human scalp hair, conducted by University of Toronto, boys under 16 years of age residing in contaminated area showed consistently higher lead levels than girls of the same age group and from the same area. The explanation for the higher lead levels in boys was not clear. It is also interesting that a similar large difference in the lead content of hair was not observed among boys and girls of same age in the control group.[95]

One influencing factor in our study was that more boys came from the Črna na Koroškem community (56 boys, 43 girls), which is more polluted of the two communities, but on the other hand there were more girls (7 girls out of 11 children) from the most polluted area (Group 12). We could not find any evidence that either sex would be more susceptible for lead uptake due to physiological reasons so we searched elsewhere. There are many factors which may influence blood lead burden, and behavior is one of them. It is different for every individual, but there are some patterns which are generally adopted – it is believed that boys spend more time playing outside on the ground and that they are not as clean as girls. More time spent outdoors and less frequent washing of hands in boys was also observed in the study of children's exposure to pesticides.[96] These factors can influence exposure to lead, but in order to draw conclusions about a larger proportion of boys with elevated blood lead burden, additional specific research of the behavior patterns would be needed. The same could be said about extreme values over 400 μg/l, which have all been observed in girls. Since there were only 3 such cases, each one should be dealt with individually in order to find out reasons for high blood lead levels and to prepare and carry out programs for improvement. Actually, by considering a relatively low absolute number of children with elevated blood lead levels, such an individual approach could be used for each of them. Like experts suggest, proper risk-reducing measures with follow-up should be implemented for these children.[97] Besides behavior, there are also some other possible exposure factors, which can be specific for each case. Paint is well known source of lead in older houses, parents occupational exposure to lead[57] dust and construction works[98] in apartment can bring additional lead rich dust in child's environment, some toys are colored with lead based paint, some jewelry can have high lead content[99] etc.. Lead uptake also depends on diet and is larger before the meal than after meal and on whether the diet is iron and calcium insufficient[77]. All these factors must be considered when searching for solutions.

A larger proportion of children with elevated blood lead levels from the Črna na Koroškem community was somehow expected since most polluted area where the smelter is located lies in Črna na Koroškem. Beside the smelter location, the predominant direction of winds also blows towards the town of Črna na Koroškem.[100] Thus, throughout history, a larger proportion of lead dust has been transmitted and deposited in the area of Črna na Koroškem than in the area of Mežica. Larger environmental pollution of environment in the community of Črna na Koroškem was confirmed with measured lead concentrations in soil and dust.[46] Measured blood leads levels attest to larger concentration of lead in environment factors in this case.

Based on the results, it can be said that the group with higher risk for elevated blood lead levels are the boys living in the community of Črna na Koroškem.

5.4.2 Comparison of the IEUBK model predictions with empirical data (groups)

It was decided to comment the results for groups were at least 5 children were included, the others were included only in calculations and results evaluation for the entire region of the study. For most groups, the values predicted by the IEUBK model were higher than values calculated from the observed data. Large differences between predicted and observed data were observed in groups 2 and 9, while results for other groups showed a satisfying level of agreement.

Blood lead values predicted by the IEUBK model for groups 3, 4, 10 and 12 showed good agreements with empirical values. 95 % confidence intervals for the IEUBK predicted and observed geometric mean blood lead levels and proportions of children with elevated blood lead levels overlapped substantially. It is important to mention that more site specific data for environmental lead was available for group 10 than for other groups. The same group also included more children than other groups, thereby making the conclusions for group 10 a bit more persuasive. For group 12, predicted values were a bit higher than the observed ones. The highest blood lead concentrations were observed in that group, which was located in the most polluted area near the smelter. That confirms the fact that higher blood lead concentrations in children reflect exposure to higher lead concentrations in the environment. On the other hand, a wide range of the measured blood lead concentrations shows that lead exposure and uptake could be influenced by each individual. Personal hygiene, child's diet, regular house cleaning, avoiding dust friendly materials (rugs, large curtains, plushy toys etc…) can play an important role in lead exposure and uptake.[14] For groups 5 and 8, differences between predicted and observed values were a bit larger. Calculated values for geometric mean of blood lead and probability that level of concern will be exceeded were a bit higher than observed values for both groups. 95% confidence intervals for proportion of children with elevated blood lead levels overlapped better than confidence intervals for blood lead geometric mean. Values calculated from the IEUBK model were much higher than values calculated from empirical blood burden data for remaining two groups with more than 5 children (groups 2 and 9). Predicted and observed values did not agree at all. According to calculations, there should be over 70% of children with elevated blood lead levels in group 9 and almost 90 % in group 2. In reality however, there was only one out of seven in group 9 and two out of twelve in group 2. Very wide 95% confidence intervals overlapped for group 9 only to a small extent, and not at all for group 2. The IEUBK model also includes a multiple-source option calculating household dust concentration from concentrations of soil lead, air lead and other sources. The default values for parameters contribution to household dust are: 70% of the soil concentration plus 100 times the air lead concentration.[66] If that option was used for group 2, calculated household dust concentration would have been much higher than measured one. The ratio between the two is higher than in any other group. A large difference between measured household dust lead concentration and dust concentration calculated by the model from soil and air lead concentrations can also be observed in group 9. In this case, a measured value is much higher than it should be according to soil lead concentration. This is interesting because a similar situation was observed in both groups with a large disagreement in predicted and measured blood lead levels. Maybe predicted blood lead levels would agree better with measured ones if there was more data available on environmental lead concentrations (more samples taken on the same locations). In general, the IEUBK predictions should be better if more site specific data was available (more samples taken on more locations).

Like in most groups, results for the whole area show that IEUBK predicted values are a bit higher than empirical ones. According to prediction of the model, 67.9 % of children should have elevated blood lead levels, which would mean 86 of 126 children included in groups, however there were only 67 such children. Mcnemar's test did not show significant differences between the IEUBK predicted and observed elevated blood lead levels in population of children included in groups (p=0.21).

5.4.3 Comparison of the IEUBK model predictions with empirical data (locations)

1000 by 1000m squares and averages were used for environmental lead concentrations in the second approach. This approach is less suitable for testing of the IEUBK model as exposure of children is described very superficially, especially in our case where the area was not well covered with sampling points for environmental factors. On the other hand, there were more children included in every group, which improves a basis for comparison.

Although this approach is less suitable for testing model as the first one, the results were quite similar. Predicted and observed values for individual groups were in good accordance for locations H11, I11 and G12. 95 % confidence intervals for the IEUBK predicted and observed geometric mean blood lead levels and proportions of children with elevated blood lead levels overlapped substantially. The highest environmental lead concentrations and highest blood lead concentrations were observed at the location where the smelter is located (G12).

Confidence intervals did not overlap at all for Location D11. For this location, predicted values are much higher than observed ones. The IEUBK model predictions for this group cannot be estimated as satisfactory. We also can not be satisfied with the agreement of predicted and observed values for Location C11. Predicted values for geometric mean of blood lead concentration and predicted proportion of children with elevated blood lead level are both substantially higher than observed values. For remaining locations (C10, I10), 95% confidence intervals for proportion of children with elevated blood lead levels overlapped better than confidence intervals for blood lead geometric mean. Calculated values for geometric mean of blood lead and a probability that level of concern will be exceeded were somewhat higher than observed values for both locations. When comparing the results for the whole area, the difference between a predicted and observed proportion of children with elevated blood lead values was higher than in the first approach. In this case, Mcnemar's test showed significant differences between the IEUBK predicted and observed elevated blood lead levels in population of children included ($p<0.01$). Larger area, including more children, was covered by this approach, but the exposure of groups of children was described worse than in the first approach.

The most comprehensive comparison between model predictions of blood lead concentrations in children and observations from epidemiologic studies of hazardous waste sites showed better agreement of results than our study. Data on environmental lead and blood lead concentrations was collected at four Superfund NPL (National Priorities List) sites. The IEUBK model predictions agreed reasonably well with observations for children who spent no more than 10 hours/week away from home. The predicted geometric mean blood lead concentrations were within 7 μg/l of the observed geometric means at each site. The predictions of the percentage of children with elevated blood lead levels were within 4% of the observed percentage at each site.[86]

The IEUBK predicted and observed blood lead values also agreed well in a study carried out in Belgium. The IEUBK model was used to predict blood lead concentrations in children living in the vicinity of a non-ferrous plant situated in Hoboken. The model was able to predict measured blood lead concentrations adequately, except for the lower exposure group. In this study, soil ingestion values and lead exposure from food were adapted to Flemish values. While changed lead exposure from food did not influence the results of the IEUBK simulations a great deal, a substantial overestimation would have been found if default soil ingestions values of the model were used.[81]

In some other studies, the results were similar to the results of our study, showing a bit larger differences between measured and predicted values.[87,88,101] In most cases, like in ours, the model over-predicted observed blood lead levels. In a study at the Bunker Hill Superfund Site in the Silver Valley of Idaho that over-prediction decreased as the environment remediation programs progressed and the exposure reduced. The agreement of predicted and observed values improved when lower constant bioavailability (18 %), and a partition rate more reflective of the site specific pathways than the default 55:45 dust:yard soil ingestion ratio, were used in the model.[101]

There was no common observation in the studies that the values predicted by the IEUBK model would better agree with the values calculated from measured blood lead levels of children living in more or less polluted areas. It is nevertheless recommended that the IEUBK model is not to be relied upon for exposure combinations leading to a predicted mean blood lead level greater than 300 μg/l because the exact nature of nonlinear relationship between exposure and blood lead is less certain in this range of blood lead levels.[86]

Despite the lack of input data, values predicted by the model agreed satisfactorily with observed values for most groups (6 out of 8) and locations (5 out of 7) in our study. In general, however, it is difficult to draw strong conclusions because of the small sample sizes. Both, the IEUBK input data and observed blood lead data, should be improved for stronger conclusions. Nevertheless, results present a good basis for further research.

We were not so concerned with the evaluation of the model itself, but focused more on the information which could be gathered when using the model with data for the Upper Meža Valley. In this aspect, our calculations showed that blood lead concentration in children living in the most polluted area are higher and that house dust and soil should be the most important exposure factors. Other two factors used in calculations (lead in drinking water, lead in air) seem to have smaller impact on blood lead level than dust and soil. When running the model in step by step mode from the main screen, it also returns prediction for lead uptake from each specific exposure factor. Our calculations revealed that soil and indoor dust contributed over 80% to overall lead uptake, while air and drinking water contributions remained under 10%.

The IEUBK models calculated uptake of lead from air is low, even if simulations are done with very high values for air lead concentrations (around 0.5 μg/m^3), recently measured on location near the smelter.[102] One study tried to evaluate risk to children from exposure to dust enriched air on sites contaminated with lead and undergoing remedial activities. The study explored modeling approaches for assessing potential risk to children from air emissions. Beside the IEUBK model, the International Commission of Radiological Protection (ICRP) model was also used to simulated blood lead concentrations in children, based on monitored air lead concentrations. Although air lead concentrations exceeded 1.5 μg/m^3, both models indicated that exposures to children in the community areas did not pose an unacceptable level of risk. [80]

In spite of these findings, it is still hard to completely ignore the role of air lead in the overall exposure of children living in the Upper Meža Valley. In addition to already mentioned recently measured much higher air lead concentrations on the locations compared to the ones used in our calculations, there are some additional facts worth mentioning. Air samples are taken about 1.5 meters above the ground, which is much higher than three-year old inhale, and because of dust redispersion from the ground, the lead concentrations in the air are probably higher closer to the ground. Another important aspect is that smaller particles have greater impact on overall blood lead burden than larger.[74] Particles smaller than 1μg are assumed to undergo greater rates of deposition and absorption in the lungs than larger particles. Particle size also influences the degree of gastrointestinal absorption. Solubility of lead sulfide in gastric acid is much greater for particles of 30 μm than particles of 100 μm diameter.[2] The IEUBK model also includes air lead fraction into household dust concentration. Regarding the above, air lead could play a more important role in overall exposure of children to lead than the IEUBK predicted uptake of lead from air indicates.

The IEUBK model considers drinking water to be a bit more important factor of exposure to lead than air, but even when the simulations were ran with the highest measured drinking water lead concentration from our data, the predicted lead uptake for this fraction did not exceed 10% of total lead uptake. In the case of the Upper Meža Valley, we can be pretty sure that lead in drinking water is not an important exposure factor for children.

Calculations with our data showed that house dust and backyard soil contribute large proportions to the overall exposure of children to lead. House dust seems to be the most important factor and if we could lower lead concentrations under a boundary immision value of 85 mg/kg, there would be over 90% of children with blood lead burden under 100 μg/l, even if lead concentrations in yard soil were around a critical immision value of 530 mg/kg. We should be very careful in making such conclusions, since yard soil is considered as the most important lead source for house dust by the IEUBK model. The cleanup strategy at the Bunker Hill Superfund Site in the Silver Valley of Idaho, based on Biokinetic pathways models, aimed to reduce house dust lead exposure through elimination of soil-borne sources. The main remedial strategy was to replace all yards having soil lead concentrations greater than 1000 mg/kg and achieve a geometric mean yard soil lead concentration of less than 350 mg/kg. It was expected that mean house dust levels will drop over 50% to less than 500 mg/kg as a result of remedial activities. During a ten-year period of remedial activities, not only house lead concentration, but also children's blood lead levels decreased significantly.[101] That example confirms that soil lead is an important source of house lead.

House dust lead was also found to be the most important factor of children's exposure in study carried out in Hoboken, Belgium. Average concentrations of house dust in that area ranged from 234-73394 mg/kg. Authors listed the following possible lead sources: the intrusion of suspended matter, being either primary emissions or secondary resuspension of outdoor soil and dust, the entrainment of outdoor dust and soil with shoes, and the intrusion of "old" dust from attics. Lead loading patterns in houses indicated an important entrainment of lead with shoes. The highest lead contamination was found in houses with unused attics where sleeping rooms immediately beneath the attic showed higher lead concentrations than the remainder of the house. That study also estimated the importance of the exposure routes to lead based on the IEUBK output files. On average, between 75% and 89% of exposure resulted from the ingestion of soil and dust for different areas. Exposure from food was from 10% to 23%, whereas exposure from the inhalation of suspended dust remained below 2%. Dust was not estimated to be a significant pathway with regard to inhalation, but could be a significant transfer route.[81]

In another comparison of the IEUBK model predictions with actual blood lead levels, authors pointed out that dust lead surface loading could be an important factor in an accurate model prediction. In homes with low levels of dust, lead concentrations may be particularly high even though little lead is present. In such cases, exposure to lead dust may be greatly overestimated when using the IEUBK model. [103]

One conclusion we can draw from our results and results of the studies mentioned above is that lead enriched dust is an important factor of exposure for children. The greatest amount of dust enters the body through ingestion[66], so the main route of exposure is not through inhalation but through ingestion. Children swallow dust. Larger part is swallowed through dirt on the food, toys and hands, which children put in their mouth. Lead may be swallowed by eating food and drinking liquids that contain it, also some lead particles that are cleared by the lung can be swallowed afterwards.

5.5 Upsides and downsides

The main advantage of our study was that we used existing data from other studies to create new knowledge about environmental lead pollution and blood lead burden of children from the Upper Meža Valley. Many different studies previously described the problems of the polluted environment and inhabitant’s health problems, but only few offered practical solutions. Some general activities on how to lower lead exposure and intake were proposed, but none of them clearly pointed out the most important exposure factors. In this aspect, our study marks a step forward. The results show that the children living in the most polluted area also have the highest blood lead burden. The results clearly indicate that house dust and soil are very important factors of lead exposure in the Upper Meža Valley.

On the other hand, carrying out study with the existing data does not allow much room in the study design. The largest limitation was the lack of environmental data to properly describe exposure of every child to lead. The consequence of that were less accurate predictions of blood lead levels by the IEUBK model. All together led to rather weak conclusions, when the IEUBK predicted geometric mean blood lead concentrations and proportions of children with elevated blood lead levels were compared with observed values.

5.6 Recommendations for future investigations

Further simulation with the IEUBK model using improved input data would be interesting. To improve input data, more samples of soil, indoor dust, air and drinking water should be taken into account and bioavailability of lead should be evaluated. Investigations about other exposure factors, like home remedies and paint, would also be interesting. A very important factor in children lead exposure is also diet lead intake, which should also be evaluated for local inhabitants. Behavior patterns of children were also followed in some others studies, to find children whose blood lead levels reflect environmental lead exposure in the best way. All that data would improve the IEUBK predictions for the area of concern. On the other hand, more blood samples of each child and averages used for comparison with the IEUBK predictions would also improve the quality of our research. With both, much more valid conclusions about the agreement between the IEUBK predicted and observed blood lead values could be made. On the other hand, the IEUBK model was validated in some extensive studies with plenty of data [86,101], so further studies should include a lot of very precise data if reasonable contribution is to be expected. We consider that it is more important to improve the ways of using the IEUBK model in order to find solutions for the Upper Meža Valley environment than studying the IEUBK model in detail. It is our belief that future studies should try to further explain important exposure factors to lead and find solutions to reduce their influence, rather than focus on validation of the IEUBK model. Gathering additional information about the IEUBK model would be a welcome side effect of such studies.

5.7 Some additional comments

The main reason of our research still remains to solve the problem of environmental lead pollutions and inhabitants' exposure to it. In this aspect, the IEUBK model is a helpful tool. In general, one of the intended uses of modeling is clarifying the process. The IEUBK model certainly helps us to better understand overall exposure of children to lead from many sources. The whole process and role of different exposure factors in it are explained. That makes it easier to find out the most important exposure factors and the ways they contribute to overall blood lead burden. In this aspect, another confirmation that house dust and soil are very important exposure factors is the most important conclusion of our study. The environmental remedial activities like covering macadam surfaces, exchanging upper soil layer, grassing of uncovered soil surfaces etc.., should be effective. By carrying out these activities, lead exposure should be lowered and blood lead levels of children should decline. The environmental remediation program was prepared[104] and is being carried out gradually. Additional studies of possible exposure factors should be carried out and strategies for the problem solution proposed. Only by closely monitoring and constantly improving the remediation program, a success can be expected.

Empirical comparisons of the IEUBK model have shown that the agreement between model predictions and observed blood lead concentrations is influenced by numerous factors including the extent to which the exposure and blood lead measurements are adequately matched (time and location), and site-specific and personal factors (e.g., soil characteristics, bioavailability, behavior patterns, individual behavior of children) that may affect lead intake or uptake in children. But even if more environmental (air, soil, dust, drinking water) samples would be taken and behavior patterns of children would be studied in detail, the model could not adequately replace blood lead samples. Results of blood lead samples show lead burden of each individual child, also taking into account child-specific susceptibility to environmental lead exposure, while the model only predicts the most probable lead burden of a typical child. It is important to find out more susceptible children and provide them with proper individual treatment. In region with a relatively small number of children, like ours, the costs of collecting enough environment data to sufficiently describe exposure of children could also be very high. In this aspect, testing blood lead samples could be less expensive than running the IEUBK model simulations for the Upper Meža Valley.

Like already said, there are a lot of other potential important exposure and uptake factors which should be studied and included in the calculations with the model if we want to come up with better results, but that could be the subject of future studies. We think that the most important factors of former lead emissions were included in our study and that the results show that blood lead burden in children is higher in places with larger environmental lead concentration.

6 CONCLUSIONS

6.1 Main conclusion

Our hypothesis was: “The IEUBK model will act well, when using data on environmental lead pollution for the Upper Meža Valley, already for available input data. The predicted values for geometric mean blood lead and proportion of children with elevated blood lead levels will be in satisfying agreement with observed values.” Considering a lack of site specific data, we can be satisfied with the accordance of values predicted by the IEUBK model with the values calculated from empirical data. The results were in good accordance for 6 out of 8 groups with at least 5 children, when the first approach was used, and for 5 out of 7 groups in the second approach. Calculated 95% confidence intervals overlapped quite well, but were too wide for strong conclusions. Predicted proportions of children with elevated blood lead level were a bit higher for most groups and also for the whole area for both approaches. We can conclude that our hypothesis was partially confirmed; the agreement of results was satisfactory for most groups, but due to lack of data this conclusion is rather weak. In terms of evaluating the precision of the IEUBK predictions, both quality of input data and quality of observed blood lead data should be improved.

6.2 Other conclusions

We can conclude that the IEUBK model is a very useful tool. This is due to the simple fact that almost any parameter included in the calculations can be changed if proper data is available. With sufficient site specific data, exposure of children can be well described and the most important factors of exposure can be determined, which is very important in preparing remediation strategies.

Another conclusion we can draw is that in our study the areas with the highest lead concentrations in yard soil and house dust also have the largest proportions of children with elevated blood lead levels.

We should not forget that the final goal of our research is to lower lead blood burden of children under level of concern. In this aspect, identifying important exposure factors, with help of the IEUBK model, is a more important part of our research. One fact we can confirm, using the calculations we performed with the IEUBK model, is that house dust and backyard soil contribute large proportions to the overall lead exposure and remediation strategies should focus on them.

7 SUMMARY

The Upper Meža Valley has mining tradition extending five centuries. In addition to mining tradition, health studies have been conducted for 50 years. Virtually all studies revealed large lead concentrations in environment factor and large lead burden in the inhabitants of the Upper Meža Valley. The Comparative Study of the Environment Pollution in the Upper Meža Valley between 1989 and 2001 is the last comprehensive study of lead and other heavy metals burden in the Upper Meža Valley in which pollution of the environment and large burden in men were confirmed again. That study also triggered the start of the project “Living with Lead”, part of which is also the testing of blood samples of 3-year old children for lead. Detected concentrations are still well over 100 μg/l.

The Integrated Exposure Uptake Biokinetic (IEUBK) Model for Lead in Children is a computer added tool for prediction of elevated blood lead levels in children under the age of seven who are exposed to environmental lead from many sources. The model estimates a plausible distribution of Pb blood concentration that is centered on the geometric mean Pb blood concentration and calculates the probability or the risk that the level of concern (≥100 μg/l) will be exceeded in children living in a polluted area.

In the study, we used data from the Comparative Study of the Environment Pollution in the Upper Meža Valley between 1989 and 2001 carried out by ERIC-o Institute in the period 2001 – 2002, and results from the project “Living with lead” which is carried out by the Regional Institute of Public Health Ravne na Koroškem. From the first one, we used data on environmental lead burden for calculations with the IEUBK model. Data on blood lead burden in children from the project “Living with lead” was merged with blood burden data from ERICOs study. We also used data on lead in drinking water from the Regional Institute of Public Health Ravne na Koroškem archives. We mapped data on environmental pollution and human blood burden and formed groups in which data on environment pollution and lead burden of children from the same geographic area (neighborhood) was merged. We also used another approach in which we created a net of 1000 by 1000 meters squares over the map of the entire area of concern. Data from the same square represented each group (location). Overlapping of 95% confidence intervals of predicted and

observed values was used to evaluate agreement of results for each group. Only results for groups which included at least five children were discussed. Mcnemar's test was applied for evaluating the results for the whole area. The chi-square test was applied to compare blood lead levels according to sex and community of residence.

The chi squre test showed that boys ($p=0.01$) and residents of the Črna na Koroškem community ($p<0.01$) are more likely to have elevated blood lead levels than girls and residents of the Mežica community. Values predicted by the IEUBK model were higher than values calculated from observed data for 7 out of 8 groups when the first approach was used. Results for most groups (6 out of 8) showed a satisfying level of agreement. Like in most groups, results for the whole area showed that IEUBK predicted values are a bit higher than empirical ones. According to prediction of the model, 67.9 % of children included in the groups should have had elevated blood lead levels, in fact only 53.2% did. That difference was not statistically significant according to Mcnemar's test (66.7% cutpoint, $p=0.21$).The results from the second approach were quite similar. Predicted and observed values for individual groups were in satisfying accordance for 5 out of 7 locations. The difference between predicted and observed proportion of children with elevated blood lead values for the whole area was a bit higher than in the first approach and was statistically significant according to Mcnemar's test (66.7 % cutpoint, $p<0.01$).

Our experiment showed that the IEUBK model is a useful tool for prediction of blood lead level in young children, but very complete and precise data on environmental lead pollution and inhabitants behavior patterns are needed.

Although our data on environmental lead concentrations was not as complete as we would like it to be, we confirmed that children with the highest blood burden do live in the most polluted areas. Another conclusion we could come up with is that soil and especially dust are the most important exposure factors. Environment remediation strategies should focus on reducing dust in children’s surroundings and lead content in it.

Key words: blood lead, children, Meža Valley, lead, uptake, exposure, modeling

8 POVZETEK

Naslov: IEUBK model za oceno koncentracije svinca v krvi otrok in njegova uporabnost pri raziskovanju in remediaciji okolja v Zgornji Mežiški dolini

Zgornja Mežiška dolina ima petsto letno rudarsko tradicijo. Poleg rudarske tradicije pa je tudi že dobrih 50 let zdravstvenih študij. Praktično vse študije so pokazale visoke koncentracije svinca v različnih faktorjih okolja in veliko obremenjenost s svincem pri prebivalcih Zgornje Mežiške doline. Primerjalna študija onesnaženosti okolja v Zgornji Mežiški dolini med stanji v letih 1989 in 2001 je zadnja obsežna študija o obremenjenosti Zgornje Mežiške doline s svincem in ostalimi težkimi kovinami, ki je znova potrdila visoko onesnaženost okolja in obremenitev ljudi. Ta študija je tudi sprožila začetek projekta "Življenje s svincem", katerega del je tudi preskušanje vzorcev krvi tri leta starih otrok na vsebnost svinca. Izmerjene koncentracije so še vedno precej nad 100 µg/l. IEUBK model za svinec pri otrocih je računalniško podprto orodje za napoved povišanih vsebnosti svinca v krvi otrok do sedmega leta starosti, ki so izpostavljeni svincu v okolju iz številnih različnih virov. Model ocenjuje verjetnostno distribucijo koncentracije svinca v krvi okoli geometrične sredine za vrednost svinca v krvi in izračunava verjetnost oziroma tveganje, da bo pri otrocih, ki na onesnaženem območju živijo, presežena vrednost (100 µg/l) pri kateri je zaradi možnih negativnih vplivov na zdravje potrebno ukrepati.

V študiji smo uporabili podatke iz Primerjalne študije onesnaženosti okolja v Zgornji Mežiški dolini med stanji v letih 1989 in 2001, ki jo je v letih 2001 - 2002 izvedel ERICo in podatke iz projekta: "Življenje s svincem", ki ga izvaja Zavod za zdravstveno varstvo Ravne na Koroškem. Iz ERICO-ve študije smo uporabili podatke o vsebnosti svinca v okolju za izračune z IEUBK modelom. Podatke o vsebnosti svinca v krvi otrok pridobljene v okviru projekta: "Življenje s svincem" smo združili s kompatibilnimi podatki iz ERICO-ve študije. Uporabili smo tudi podatke o vsebnosti svinca v pitni vodi iz arhiva Zavoda za zdravstveno varstvo Ravne. Podatke o onesnaženosti okolja in obremenjenosti s svincem pri ljudeh smo vnesli na geografsko karto in na podlagi bližine lokacije oblikovali skupine, v katerih smo združili posamezne podatke o koncentracijah svinca v okolju in koncentracijah svinca v krvi otrok. Uporabili smo tudi drugi pristop pri katerem smo na karti oblikovali mrežo kvadratov 1000 krat 1000 metrov preko celotnega območja, ki je bilo predmet raziskave. Podatki, ki so bili znotraj istega kvadrata, so predstavljali eno skupino (lokacijo). Ujemanje napovedanih in empiričnih vrednosti je bilo ocenjeno na podlagi prekrivanja 95% intervalov zaupanja obeh vrednosti. Podrobneje smo obravnavali samo rezultate za skupine v katere je bilo uvrščenih vsaj 5 otrok. Za oceno rezultatov za celotno območje je bil uporabljen McNemarjev test. Za primerjavo vsebnosti svinca v krvi, glede na spol in občino bivanja, je bil uporabljen hi-kvadrat test.

Hi-kvadrat test je pokazal, da je verjetnost za povišane vsebnosti svinca v krvi večja pri dečkih ($p=0.01$) in občanih Črne ($p<0.01$), kot pa pri deklicah in občanih Mežice. Ob uporabi prvega pristopa, so bile vrednosti, ki jih je napovedal IEUBK model, nekoliko višje od vrednosti izračunanih iz dejansko izmerjenih vsebnosti svinca v krvi otrok, pri 7 izmed 8 skupin. Za večino skupin (6 izmed 8) je bila skladnost rezultatov zadovoljiva. Podobno kot za večino skupin so bili tudi rezultati izračunov modela IEUBK za celotno obravnavano območje nekoliko višji od izmerjenih. Model je napovedal, da bi moralo imeti povišane vsebnosti svinca v krvi 67,9% otrok, dejansko pa je imelo povišane vsebnosti le 53,2% otrok. Glede na McNemarjev test ta razlika ni bila statistično značilna (66% ločnica,$p=0.21$) Pri drugem pristopu so bili rezultati podobni, kot pri prvem. Vrednosti, ki jih je napovedal model, so pokazale zadovoljivo skladnost z izmerjenimi vrednostmi za 5 izmed 7 lokacij, medtem ko je bila razlika med napovedanim in dejanskim deležem otrok s povišanimi vsebnostmi svinca za celotno območje nekoliko višja, kot pri prvem pristopu in je bila, glede na McNemarjev test (66% ločnica,$p<0.01$) statistično značilna.

Naš poskus je pokazal, da je IEUBK model uporabno orodje za napoved vsebnosti svinca v krvi otrok, vendar potrebujemo celovite in natančne podatke o onesnaženosti okolja s svincem in vzorcih obnašanja prebivalstva. Čeprav naši podatki o onesnaženosti okolja niso bili tako celoviti, kot bi si želeli, smo potrdili, da otroci z najvišjimi vsebnostmi svinca živijo na najbolj onesnaženih območjih.

Zaključimo lahko tudi, da sta zemlja in še posebno prah najpomembnejša faktorja izpostavljenosti. Strategije sanacije okolja bi bilo potrebno usmeriti v zmanjšanje količine prahu v bivalnem okolju otrok in vsebnosti svinca v tem prahu.

Ključne besede: svinec v krvi, otroci, Mežiška dolina, svinec, sprejem v telo, izpostavljenost, modeliranje

9 REFERENCES

1. Wikipedia contributors. "Lead" Wikipedia, The Free Encyclopedia. http://en.wikipedia.org/w/index.php?title=Lead&oldid (4.Apr. 2007).
2. Agency for Toxic Substances and Disease Registry (ATSDR).2005. Toxicological Profile for Lead. Atlanta, GA. U.S. Department of Health and Human Services, Public Health Service: 577pag.
3. IPCS. 1995. Environmental Health Criteria 165: Inorganic Lead. International Programme on Chemical Safety. World Health Organization. Geneva: 300 pag.
4. Likar M. 1998. Vodnik po onesnaževalcih okolja. Ljubljana, Zbornica sanitarnih tehnikov in inženirjev Slovenije: 312-318.
5. Surovine.si. Indsutrijske kovine, svinec http://www.surovine.si/svinec.php (26.Apr.2008)
6. Freeman N., Sheldon L.,Jimenez M.,Melnyk L.,Pellizzari E., Berry M. 2001. Contribution of children’s activities to lead contamination of food. Journal of Exposure Analysis and Environmental Epidemiology, 11: 407–413.
7. Melnyk L., Berry M., Sheldon L., Freeman N., Pellizzari E., Kinman R. 2000. Dietary exposure of children in lead-laden environments. Journal of Exposure Analysis and Environmental Epidemiology,10:723–731.
8. Maas R. , Patch S.C., Pandolfo T.J., Druhan J.L., Gandy N.F. 2005. Lead content and exposure from children's and adult's jewelry products. Bulletin of Environmental Contamination and Toxicology, 74:437-444.
9. Moore M., Meredith P., Watson W., Sumner D., Taylor M., Goldberg A. 1980. The percutaneous absorption of lead-203 in humans from cosmetic preparations containing lead acetate, as assessed by whole-body counting and other techniques. Food and Cosmetics Toxicology, 18:399-405.
10. Chiaradia M., Gulson B. and MacDonald K. 1997. Contamination of houses by workers occupationally exposed in a lead-zinc-copper mine and impact on blood lead concentrations in the families. Occupational and Environmental Medicine, 54, 2:117-124.
11. World Health Organization. 2000. Air quality guidelines for Europe, 2nd ed. Chapter 6.7 Lead. Copenhagen, Regional Office for Europe. http://www.euro.who.int/document/aiq/6_7lead.pdf (2.May 2008)
12. Ito Y.,Niiya Y.,Otani M.,Sarai S., Shima S. 1987. Effect of food intake on blood lead concentration in workers occupationally exposed to lead. Toxicology Letters, 37:105-114.
13. Rabinowitz M., Kopple J. and Wetherill G. 1980. Effect of food intake and fasting on gastrointestinal lead absorption in humans. Americal Journal of Clinical Nutrition, 33:1784-1788.
14. Mandl N., Janet E., Horvat M., Kašnik Janet M., Ivartnik M., Pavlič H. 2006. Promocija zdravja v lokalni skuponosti v zvezi z zmanjševanjem izpostavljenosti svincu. Cvahtetovi dnevi javnega zdravja 2006, Ljubljana, 9.jun.2006. Bilban M. Ljubljana, Medicinska fakulteta: 133-141.
15. Horvat M., Janet E. in Ivartnik M. 2007. Svinec v krvi triletnikov iz Mežiške doline. Cvahtetovi dnevi javnega zdravja 2007, Ljubljana, 28. Sep 2007. Zaletel Kragelj L. Ljubljana, Medicinska fakulteta: 16-24.
16. Gulson B., Mizon K., Korsch M., Palmer J., Donnelly J. 2003. Mobilization of lead from human bone tissue during pregnancy and lactation—a summary of long-term research. Science of Total Environment, 303:79–104.

17. American Academy of Pediatrics: Committee on Environmental Health. 2005. Lead Exposure in Children: Prevention, Detection, and Management. Pediatrics, 116: 1036-1046. http://pediatrics.aappublications.org/cgi/reprint/116/4/1036?maxtoshow=&HITS=10&hits=10&RESULTFORMAT=&fulltext=lead&searchid=1&FIRSTINDEX=0&sortspec=relevance&resourcetype=HWCIT (29. Mar. 2006)
18. Weisskopf M., Wright R., Schwartz J., Spiro A., Sparrow D., Aro A., Hu H. 2004. Cumulative Lead Exposure and Prospective Change in Cognition among Elderly Men. American Journal of Epidemiology, 160,12: 1184-1193. http://aje.oxfordjournals.org/cgi/content/full/160/12/1184 (12. Feb. 2008)
19. Jain N., Laden F., Guller U., Shankar A., Kazani S., Garshick E. 2005. Relation between Blood Lead Levels and Childhood Anemia in India American Journal of Epidemiology, 161,10: 968 - 973.
20. Winder C. 1993. Lead, reproduction and development. Neurotoxicology, 14, 2-3:303-318.
21. IARC. 2006 IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Vol. 87. Inorganic and Organic Lead Compounds. Lyon, WHO Press: 519 pag.
22. Steenland K., Selevan S. and Landrigan P. 1992. The mortality of lead smelter workers: an update. American Journal of Public Health, 82,12: 1641–1644.
23. Hu H., Aro A., Payton M., Korrick S., Sparrow D.,Weiss S.T., Rotnitzky A. 1996. The relationship of bone and blood lead to hypertension: The normative aging study. Journal of the American Medical Association, 275, 15:1171-1176.
24. Lin J., Tan D., Ho H., Yu C. 2002. Environmental lead exposure and urate excretion in the general population. The American Journal of Medicine, 113, 7: 563 – 568.
25. Sakai T. and Morita Y. 1996. δ-Aminolevulinic acid in plasma or whole blood as a sensitive indicator of lead effects, and its relation to the other heme-related parameters. International Archives of Occupational and Environmental Health, 68, 2: 126-132.
26. Ettinger A., Tellez – Rojo M., Amarasiriwardena C., Bellinger D., Peterson K., Schwartz J., Hu H. Hernandez-Avila M. 2004. Effect of Breast Milk Lead on Infant Blood Lead Levels at 1 Month of Age. Environmental Health Perspectives, 112,14: 1381-1385.
27. Ziegler E., Edwards B., Jensen R., Mahaffey K., Fomon S. 1978. Absorption and retention of lead by infants. Pediatric Research, no. 12:29–34.
28. Willes R.F., Lok E., Truelove J.F., Sundaram A. 1977. Retention and Tissue Distribution of $^{210}Pb(NO_3)_2$ Administered Orally to Infant and Adult Monkeys. Journal of Toxicology and Environmental Health, 3: 395-406.
29. Gump B., Stewart P.,Reihman J., Lonky E., Darvill T., Parsons P., Granger D. 2008. Low-Level Prenatal and Postnatal Blood Lead Exposure and Adrenocortical Responses to Acute Stress in Children. Environmental Health Perspectives,116, 2: 249-255.
30. Papanikolaou N., Hatzidaki E., Belivanis S., Tzanakakis G., Tsatsakis A. 2005. Lead toxicity update. A brief review. Medical Science Monitor, 11,10:329-336.
31. World Health Organization. 1995. Lead and health. Local authorities, health and environment briefing pamphlet Series. Copenhagen, Regional Office for Europe.
32. Lanphear B., Hornung R., Ho M., Howard C., Eberle S., Knauf K. 2002. Environmental lead exposure during early childhood. The Journal of Pediatrics, 140,1: 40-47.

33. Téllez-Rojo M., Bellinger D., Arroyo-Quiroz C., Lamadrid-Figueroa H., Mercado-García A., Schnaas-Arrieta L., Wright R., Hernández-Avila M., Hu H., 2006. Longitudinal Associations Between Blood Lead Concentrations Lower Than 10 μg/dL and Neurobehavioral Development in Environmentally Exposed Children in Mexico City. Pediatrics, 118, 2: 323-330. http://www.pediatrics.org (23. Sep. 2008)
34. Centers for Disease Control and Prevention. 2005. Preventing Lead Poisoning in Young Children. Atlanta.CDC: 77 pag. http://www.cdc.gov/nceh/lead/publications/PrevLeadPoisoning.pdf (8.Mar. 2008)
35. Schwartz J. 1994. Low-level lead exposure and children's IQ: a meta-analysis and search for a threshold. Environmental Research, 65, 1:42–55.
36. Jakubowski M. 2006. Development of a coherent approach to human Biomonitoring in Europe. Utility and sensitivity of biomarkers: 113 pag.
37. Centers for Disease Control and Prevention. Why not change the blood lead level of concern at this time? http://www.cdc.gov/nceh/lead/faq/changebll.htm (7.Oct.2008)
38. Rothenberg S. 2008. Blood Lead Levels in Children. Environmental Health Perspectives, 116,11: 472-473. http://www.ehponline.org/docs/2008/11636/letter.html (24.Jul. 2009)
39. Jobanputra N., Jones R., Buckler G., Cody R., Gochfeld M., Matte T., Rich D., Rhoads G. 1998. Accuracy and Reproducibility of Blood Lead Testing in Commercial Laboratories. Archives of Pediatrics & Adolescent Medicine, 152: 548-553.
40. Bonnefoy C., Menudier A., Moesch C., Lachâtre G., Mermet J.-M. 2002. Validation of the determination of lead in whole blood by ICP-MS. Journal of Analytical Atomic Spectrometry, 17: 1161-1165.
41. Kafourou A., Touloumi G., Makropoulos V., Loutradia A., Papanagiotou A., Hatzakis A. 1997. Effects of lead on the somatic growth of children. Archives of Environmental Health, 52, 5, 377-382.
42. Grosse S., Matte T., Schwartz J., Jackson R. 2002. Economic gains resulting from the reduction in children's exposure to lead in the United States. Environmental Health Perspectives, 110, 6:563-569. http://www.ehponline.org/members/2002/(17.Oct. 2007)
43. Fewtrell L., Pruss-Ustun A., Landrigan P., Ayuso-Mateos J. 2004. Estimating the global burden of disease of mild mental retardation and cardiovascular diseases from environmental lead exposure. Environmental Research, 94: 120- 133.
44. Uran S., Mežnar F. 1965. 300 let mežiški rudniki. Mežica, Društvo rudarskih, metalurških in geoloških inženirjev in tehnikov: 262 str.
45. Prpič- Majić D., Fugaš M., Souvent P., Sušnik J., Šarič M. 1996. Istraživanja olova, kadmija i cinka u dolini rijeke Meže. Zagreb, Inštitut za medicinska istraživanja i medicinu rada: 151 str.
46. Ribarič-Lasnik C., Eržen I., Kugonič N., Pokorny B., Končnik D., Svetina M., Justin B., Druks P., Bole M., Rošer-Drev A., Vetrih M., Flis J., Kotnik K., Mavsar R., Pačnik L., Savinek K. 2002. Primerjalna študija onesnaženosti okolja v zgornji Mežiški dolini med stanji v letih 1989 in 2001: Končno poročilo. ERICo Velenje, Inštitut za ekološke raziskave.
47. Zgodovina Mežiškega Rudnika. http://www.podzemljepece.com (13.Sep.2007)

48. Dolenc B., Košir J. 1962. Saturizem otrok v Mežiški dolini. Simpozij o saturizmu, Slovenj Gradec 1962.
49. Beritić T. 1952. Izveštaj o terenskem istraživanju u rudniku i topionici olova u Mežicama. Zagreb, Institut za higienu rada Jugoslovanske akademije znanosti I umetnosti.
50. Fugaš M. 1977. Biological significance of some metals as air pollutants. Part I Lead. Environmental Health Effects Research Series EPA-600/1-77-041. Research Triangle Park, US Environmetal Protection Agency.
51. Graovac-Leposavić L. 1975. Rezultati ispitivanja radnika topionice. Beograd, Institut za medicinu rada i radiološku zaštitu "Dr. Dragomir Karajović".
52. Prpić-Majić D. 1982. Povečana apsorpcija olova u radnika, pomočnog i tehničkog osoblja "OZD Rudniki svinca in topilnica Mežica". Izvještaj IMI-P-47. Zagreb, Institut za medicinska istraživanja i medicinu rada.
53. Jezernik B. 1987. Analiza stanja zastrupljenosti s svincem pri delavcih Rudnika Mežica in uvedba sodobnega biološkega monitoringa za spremljanje saturizma. Ljubljana, Univerza v Ljubljani: 34 str.
54. Milić M. 1976. Biotoksikološki parametri pri različitim profesionalnim ekspozicijama olovu i ekspozicijama stanovništa Mežiške doline. Beograd, Univerzitet u Beogradu: 135 str.
55. Graovac-Leposavić L., Đurić D., Valjarevič V.1973. Environmental lead contamination of Meža Valley study on lead exposure of population. V: Proceedings of the International Symposium Environmental Health Aspects of Lead, Amsterdam 1972. Luxemburg, Commission of the European Communities: 685-705.
56. Prpić-Majić D. 1978. Study of children`s blood lead levels within families. Environmental Health Effects Research Series EPA-600/1-78-067. Research Triangle Park, US Environmetal Protection Agency.
57. Prpić-Majić D., Pongračič J., Keršanc A., Telišman S., Karačič V. 1979. Increased lead absorption in children in the vicinity of a lead smelting plant. Arhiv za higijenu rada i toksikologiju, 30:151-156.
58. Prpić-Majić D., Fugaš Z.1981. Praćenje toka normalizacije nalaza bioloških pokazatelja izloženosti olovu u stanovnika oko talionice olova nakon postavljanja novih vrećastih filtara u odnosu na razine olova u okolini. Izvještaj IMI-P-35. Zagreb, Institut za medicinska istraživanja i medicinu rada.
59. Prpić-Majić D. 1987. Praćenje toka normalizacije nalaza bioloških pokazatelja izloženosti olovu u stanovnika oko talionice olova nakon postavljanja novih vrećastih filtara u odnosu na razine olova u okolini. Izvještaj IMI-P-109.Zagreb, Institut za medicinska istraživanja i medicinu rada.
60. Sušnik J. 1986. Kako zelena je (bila) moja dolina - ekološki problemi pokrajine med Peco in Pohorjem. V: Med Peco in Pohorjem 3. Mrdavšič J.(ur.). Ravne na Koroškem. Koroška osrednja knjižnica dr. Franc Sušnik: 71-89.
61. Stanković M., Milić S., Đurić D., Stanković B. 1977. Cadmium, lead and mercury content of human scalp hair in relation to exposure. V: Clinical Chemistry and Chemical Toxicology of Metals. Brown S.(ur.). Amsterdam, Elsevier: 327-331.
62. Đurić D., Kerin Ž., Graovac-Leposavić L., Novak L., Kop M. 1971. Environmental contamination by lead from a mine and smelter. A preliminary report. Archives of Environmental Health, 23: 275-279.

63. Fugaš M., Hršak J., Šisernik M. 1979-1986. Praćenje koncentracije olova, cinka i kadmija u okolici talionice olova. Izvještaji 1979-1986.Zagreb, Institut za medicinska istraživanja i medicinu rada.
64. Hršak J., Balagović I., Šisernik M. 1987-1991. Praćenje koncentracije olova, cinka i kadmija u okolici talionice olova. Izvještaji 1987-1991.Zagreb, Institut za medicinska istraživanja i medicinu rada.
65. Lapajne V., Brumen S., Medved M. 1986. Kemijske preiskave zemljin in sočivja na vsebnost svinca in kadmija na področju Žerjava. Poročilo.Maribor, Zavod za zdravstveno varstvo Maribor, Center za varstvo okolja
66. U.S. Environmental Protection Agency. 1994. Guidance Manual for the Integrated Exposure Uptake Biokinetic Model for Lead in Children. Washington DC. Office of Emergancy and Remedial Response. http://www.epa.gov/superfund/programs/lead/products.htm (8. Dec. 2006)
67. U.S. EPA. 2002. Short sheet: Overview of the IEUBK Model for Lead in Children. Washington DC, EPA Office of Emergancy and Remedial Response. http://www.epa.gov/superfund/lead/guidance.htm#leadinchildren (1. Dec. 2006)
68. von Lindern I., von Braun M. 2006. Hazardous Waste Cleanup in the Far West (Idaho, USA) and the Far East (Russia). Slovenia and US Workshop on Environmental Science & Engineering, Ljubljana. 27-30 sept. 2006. (personal source, Sep. 2006)
69. Manins, P., Allan, R., Beer, T., Fraser, P., Holper, P., Suppiah, R. and Walsh, K., 2001.Atmosphere, Australia State of the Environment Report 2001 (Theme Report), CSIRO Publishing on behalf of the Department of the Environment and Heritage, Canberra. http://www.environment.gov.au/soe/2001/publications/theme-reports/ atmosphere /pubs/atmosphere.pdf (20. Apr. 2008)
70. Pennington, J. A. T. 1983. Revision of the total diet study food list and diets. Journal of the American Dietetic Association, 82, 2: 166-173.
71. Wilhelm M. , Wittsiepe J., Schrey P., Feldmann C., Idel H. 2003. Dietary intake of lead by children and adults from Germany measured by the duplicate method.International Journal of Hygiene and Environmental Health, 206, 6: 493-503.
72. de Winter-Sorkina R., Bakker M., van Donkersgoed G., van Klaveren J. 2003. Dietary intake of heavy metals (cadmium, lead and mercury) by the Dutch population, Rijksinstituut voor Volksgezondheid en Milieu RIVM http://www.rivm.nl/bibliotheek/rapporten/320103001.html (2.May 2008)
73. Marcus, A. H., Holtzman, A. P. 1989. Relationships between infant blood lead concentrations and lead in water or liquid diet. Report from Battelle Columbus Division to U.S. EPA Office of Drinking Water.
74. U.S. Environmental Protection Agency. 1989. Review of the National Ambient Air Quality Standards for Lead: Exposure Analysis Methodology and Validation. U.S. EPA Office of Air Quality Planning and Standards.
75. Laidlaw M.,Mielke H.,Filippelli G.,Johnson D., Gonzales C. 2005. Seasonality and Children’s Blood Lead Levels: Developing a Predictive Model Using Climatic Variables and Blood Lead Data from Indianapolis, Indiana, Syracuse, New York, and New Orleans, Louisiana (USA). Environmental Health Perspectives 113:793–800. http://www.ehponline.org/members/2005/7759/7759.pdf (4. May 2008)

76. Ryu J., Ziegler E., Nelson S., Fomon S. 1983. Dietary intake of lead and blood lead concentration in early infancy. American Journal of Diseases of Children, 137: 886-891.
77. U.S. Environmental Protection Agency. 2007. Estimation of relative bioavailability of lead in soil and soil-like materials using in vivo and in vitro methods. Office of Solid Waste and Emergency Response. Washington.
78. Barry, P. S. I. 1981. Concentrations of lead in the tissues of children. British Journal of Industrial Medicine ,38: 61-71.
79. Pravilnik o obratovalnem monitoringu pri vnosu nevarnih snovi in rastlinskih hranil v tla. Ur.l. RS, št. 55-2869/97.
80. Khoury G.A., Diamond G.L. 2003. Risks to children from exposure to lead in air during remedial or removal activities at Superfund sites: A case study of the RSR lead smelter Superfund site. Journal of Exposure Analysis and Environmental Epidemiology, 13: 51 – 65.
81. Cornelis C. ,Berghmans P., van Sprundel M., Van der Auwera J. 2006. Use of the IEUBK Model for Determination of Exposure Routes in View of Site Remediation. Human and Ecological Risk Assessment, 12: 963–982.
82. Harley N., Kneip T. 1985. An integrated metabolic model for lead in humans of all ages. Final report to the U.S. Environmental Protection Agency. New York University Department of Environmental Medicine.
83. Mallon, R.P. 1983. A metabolic model of lead kinetics based upon measured organ burdens during chronic exposure experiments with infant and juvenile baboons. New York, New York University Medical Center, Institute of Environmental Medicine.
84. Lewis and Clark County Health Department, Montana Department of Health and Environmental Sciences, U.S. Department of Health and Human Services, and U.S. Environmental Protection Agency. 1986. East Helena, Montana: Child Lead Study, Summer 1983. Final Report.
85. Marlin H. Mickle. 1998. Structure, Use, and Validation of the IEUBK Model Department of Electrical Engineering, University of Pittsburgh, Pittsburgh. Pennsylvania. Environmental Health Perspectives, 106, 6.: 1531-1534.
86. Hogan K., Marcus A., Smith R.,White P. 1998. Integrated Exposure Uptake Biokinetic Model for Lead in Children: Empirical Comparisons with Epidemiologic Data. Environmental Health Perspectives, 106,6:1557-1567. http://www.ehponline.org/members/1998/Suppl-6/toc.html (10.Apr.2008)
87. The National Academy of Sciences. 2005. Report on Superfund and Mining Megasites: Lessons from the Coeur d´Alene River Basin. Washington, The National Academy of Sciences: 504 pag. http://www.epa.gov/superfund/reports/pdfs/coeur/coeur.pdf (11. Dec. 2006)
88. Biesiada M. and Hubicki L. 1999. Blood Lead Levels in Children: Epidemiology vs. Simulation. European Journal of Epidemiology, 15,5: 485-491.
89. Bowen H. J. M. 1979. Environmental Chemistry of the Elements. London. Academic Press: 333 pag.
90. Šajn R. 2002. Vpliv rudarjenja in metalurške dejavnosti na kemično sestavo tal in podstrešnega prahu v Mežiški dolini. Geologija, 45,2: 547–552. http://www.geo-zs.si/publikacije_arhiv/Clanki/Geologija_45_2/(27.Apr.2008)
91. Uredba o mejnih, opozorilnih in kritičnih imisijskih vrednostih nevarnih snovi v tleh. Ur.l.RS št. 68-3722/96

92. White P. 1998. The Conceptual Structure of the Integrated Exposure Uptake Biokinetic Model for Lead in Children. Environmental Health Perspectives,106, 6 : 1513-1530.
http://www.ehponline.org/members/1998/Suppl-6/1513-1530white/white-full.html (11. Dec. 2008)
93. Virginia department of healt. 2006. 2005 annual report summary of surveillance data for Virginia children with elevated blood lead levels Office of epidemiology. Division of public health toxicology.
http://www.vdh.state.va.us/epidemiology/publichealthtoxicology/documents/ (8.Apr. 2008)
94. Nuwayhid I., Nabulsi M., Muwakkit S., Kouzi S., Salem G., Mikati M., Ariss M. 2003. Blood Lead Concentrations in 1–3 Year Old Lebanese Children: A Cross-sectional study Environmental Health: A Global Access Science Source.
http://www.ehjournal.net/content/2/1/5 (4.Apr.2008)
95. Chattopadhyay A., Roberts T.M., Jervis R.E. 1977. Scalp Hair as a Monitor for Community Exposure to Lead. Archives of Environmental Health, 32 (5): 226- 235.
96. Freeman N., Jimenez M., Reed K., Gurunathan S., Edwards R., Roy A., Adgate J., Pellizzari E., Quackenboss J., Sexton K., Lioy P. 2001. Quantitative analysis of children's microactivity patterns: The Minnesota Children's Pesticide Exposure Study. Journal of Exposure Analysis and Environmental Epidemiology, 11: 501–509.
http://www.nature.com/jes/journal/v11/n6/full/7500193a.html (3.May 2008)
97. I.Eržen, E. Janet. 2005. Svinec v krvi tri leta starih otrok, ki živijo na območju Zgornje in Spodnje Mežiške doline, Zdravstveno Varstvo, 44: 18-25.
98. Farfel M., Orlova A., Lees P., Rohde C., Ashley P., Chisolm J. 2003. A Study of Urban Housing Demolitions as Sources of Lead in Ambient Dust:Demolition Practices and Exterior Dust Fall. Environmental Health Perspectives, 111: 1228–1234.
http://dx.doi.org/(4.May 2008)
99. Berkowitz S., Tarrago R. 2006. Acute Brain Herniation From Lead Toxicity. Pediatrics, 118: 2548-2551.
100. Špes M. Vpliv lokalnih virov emisij in čezmejnega prenašanja onesnaženega zraka na kakovost okolja v alpskem ekosistemu slovenije.
http://www.ff.uni-lj.si/oddelki/geo/publikacije/dela/files/ (6.May 2008)
101. von Lindern I., Spalinger S.,Petroysan V., von Braun M. 2003. Assessing remedial effectiveness through the blood lead:soil/dust lead relationship at the Bunker Hill Superfund Site in the Silver Valley of Idaho. The Science of the Total Environment, 303: 139–170.
102. Rezultati trimesečnega monitoringa zraka v zgornji Mežiški dolini
http.//www.mop.gov.si/si/splosno/novice/novica/browse/3/article (22.Jan.2008)
103. Lewandowski T.A., Forslund B.L. 1994. Comparison of IEUBK model predictions and actual blood lead values at a former battery recycling site. Environmental Geochemistry and Health, 16,3-4: 217-222.
104. Odlok o območjih največje obremenjenosti okolja in o programu ukrepov za izboljšanje kakovosti okolja v Zgornji Mežiški dolini. Ur.l. RS, št. 119-5993/2007

ACKNOWLEDGMENT

I would like to express my gratitude to my mentor, Dr. Ivan Eržen, for guiding me in the right direction and for helping me understand some scientific work basics. He was always able to find a seemingly unimportant minor thing, which made me think and understand that there is much more knowledge to be gained and plenty of opportunities for possible research in this field still awaits. I would also like to thank my professors at the University of Nova Gorica for showing me that the useful use of knowledge is at least as important as gaining it. I must not forget my boss and other co-workers for providing me with motivation and encouragement. I would also like to thank my family for the support they have given me throughout my entire life and in particular, I must acknowledge my partner Mojca and my daughter Tinka for showing me that regardless of what I will be doing in my life there are more important things waiting for me just besides me.

ANNEX A:

Default values for the IEUBK model input parameters[61]

PARAMETER	DEFAULT VALUE
AIR	
Outdoor air lead concentration	AIR (constant) - 0.10 μg/m^3 AIR (by year) 0-7 year (0-84 mo) - 0.10μg/m^3
Ratio of indoor to outdoor air lead concentration	30 %
Time outdoors	Age = 0-1 year (0-11 mo), 1 h/day 1-2 years (12-23 mo), 2 h/day 2-3 years (24-35 mo), 3 h/day 3-7 years (36-83 mo), 4 h/day
Ventilation rate	Age = 0-1 year (0-11 mo), 2 m^3/day 1-2 years (12-23 mo), 3 m^3/day 2-5 years (24-59 mo), 5 m^3/day 5-7 years (60-84 mo), 7 m^3/day
Lung absorption	32 %
DIET	
Dietary lead intake	Age = 0-1 year (0-11 mo), 5.53 μg Pb /day 1-2 years (12-23 mo), 5.78 μg Pb /day 2-3 years (24-35 mo), 6.49 μg Pb /day 3-4 years (36-47 mo), 6.24 μg Pb /day 4-5 years (48-59 mo), 6.01 μg Pb /day 5-6 years (60-71 mo), 6.34 μg Pb /day 6-7 years (72-84 mo), 7.00 μg Pb /day
Alternate diet sources (by food class)	home-grown fruits 0 μg Pb/g (%) home-grown vegetables 0 μg Pb/g (%) fish from fishing 0 μg Pb/g (%) game animals from hunting 0 μg Pb/g (%)
Drinking water	4 μg/l
Drinking water ingestion rate	Age = 0-1 year (0-11 mo), 0.20 liters/day 1-2 years (12-23 mo), 0.50 liters/day 2-3 years (24-35 mo), 0.52 liters/day 3-4 years (36-47 mo), 0.53 liters/day 4-5 years (48-59 mo), 0.55 liters/day 5-6 years (60-71 mo), 0.58 liters/day 6-7 years (72-84 mo), 0.59 liters/day
Alternate drinking water sources	first-draw water 4 mg/l, 50% of total intake flushed water 1 mg/l, 35 % of total intake fountain water 10 mg/l, 15% of total intake
SOIL AND DUST	
Soil concentration	200 μg/g
Dust concentration	200 μg/g
Soil ingestion as percent of total soil and dust ingestion	45 %
Soil/dust ingestion	Age = 0-1 year (0-11 mo), 0.085 g/day 1-4 years (12-47 mo), 0.135 g/day

	4-5 years (48-59 mo), 0.100 g/day *5-6 years (60-71 mo), 0.090 g/day* *6-7 years (72-84 mo), 0.085 g/day*
soil/dust multiple source analysis	
Ratio of dust lead concentration to soil lead concentration	*0.70 unitless*
Ratio of dust lead concentration to outdoor air lead concentration	*100 μg Pb/g dust per μg Pb/m*^{3} *air*
soil/dust multiple source analysis with alternative household sources	
household dust	*150 μg/g (100% minus all other)*
secondary occupational dust	*1200 μg/g (0%)*
school dust	*200 μg/g (0%)*
daycare center dust	*200 μg/g (0%)*
second home	*200 μg/g (0%)*
interior lead-based paint	*1200 μg/g (0%)*
BIOAVAILABILITY FOR ALL GUT ABSORPTION PATHWAYS	
Total lead absorption at low intake	
diet, drinking water	*50 %*
soil, dust	*30 %*
alternate source	*0 %*
Fraction of lead absorbed passively at high intake	
diet, drinking water, soil, dust, alternate source	*0,2 unitless*
MATERNAL-TO-NEWBORN LEAD EXPOSURE	*Mother's blood lead level at time of birth 2.5 μg/dL*
PLOTTING AND RISK ESTIMATION	
Geometric standard deviation for blood lead, GSD	*1.6 unitless*
Blood lead level of concern, or cutoff	*10 μg/dL*
COMPUTATION OPTIONS	*Iteration time step for numerical integration 4h*

ANNEX B:

Blood lead levels in children – rank list

Rang	*Group*	*Location*	*ID no*	*Sex*	*Town*	*BB (μg/L)*
1	*Group5*	*C10*	*82*	*Female*	*MEŽICA*	*10*
2	*Group9*	*I11*	*19*	*Male*	*ČRNA*	*15*
3	*X*	*C9*	*157*	*Female*	*MEŽICA*	*16*
4	*X*	*B11*	*84*	*Male*	*MEŽICA*	*19*
5	*X*	*K14*	*165*	*Female*	*ČRNA*	*20*
6	*Group2*	*D11*	*124*	*Female*	*MEŽICA*	*21*
7	*Group5*	*C10*	*67*	*Male*	*MEŽICA*	*23*
8	*Group5*	*C10*	*155*	*Female*	*MEŽICA*	*24*
9	*X*	*C11*	*26*	*Female*	*MEŽICA*	*25*
10	*Group5*	*C10*	*121*	*Male*	*MEŽICA*	*25*
11	*Group2*	*D11*	*56*	*Female*	*MEŽICA*	*26*
12	*Group3*	*D11*	*119*	*Male*	*MEŽICA*	*27*
13	*Group8*	*I10*	*70*	*Female*	*ČRNA*	*28*
14	*X*	*I1*	*139*	*Female*	*ČRNA*	*29*
15	*Group9*	*I11*	*33*	*Male*	*ČRNA*	*30*
16	*Group7*	*D11*	*21*	*Male*	*MEŽICA*	*32*
17	*Group1*	*E11*	*116*	*Female*	*MEŽICA*	*33*
18	*Group4*	*C11*	*118*	*Female*	*MEŽICA*	*34*
19	*Group9*	*I11*	*138*	*Female*	*ČRNA*	*36*
20	*Group9*	*I11*	*134*	*Female*	*ČRNA*	*37*
21	*Group2*	*D11*	*59*	*Female*	*MEŽICA*	*38*
22	*X*	*H10*	*131*	*Female*	*ČRNA*	*38*
23	*Group3*	*D11*	*95*	*Female*	*MEŽICA*	*39*
24	*X*	*H14*	*71*	*Male*	*ČRNA*	*40*
25	*X*	*C11*	*180*	*Female*	*MEŽICA*	*40*
26	*Group10*	*I10*	*23*	*Male*	*ČRNA*	*42*
27	*Group3*	*D11*	*54*	*Male*	*MEŽICA*	*43*
28	*Group5*	*C10*	*115*	*Female*	*MEŽICA*	*43*
29	*Group4*	*D11*	*29*	*Male*	*MEŽICA*	*44*
30	*Group9*	*I11*	*101*	*Female*	*ČRNA*	*44*
31	*Group2*	*D11*	*18*	*Male*	*MEŽICA*	*45*
32	*Group5*	*C10*	*181*	*Female*	*MEŽICA*	*45*
33	*Group5*	*C10*	*37*	*Female*	*MEŽICA*	*46*
34	*X*	*J2*	*172*	*Female*	*ČRNA*	*46*
35	*X*	*C11*	*50*	*Male*	*MEŽICA*	*48*
36	*Group3*	*D11*	*65*	*Male*	*MEŽICA*	*48*
37	*Group5*	*C10*	*102*	*Male*	*MEŽICA*	*49*
38	*Group8*	*I10*	*128*	*Male*	*ČRNA*	*49*
39	*Group3*	*D11*	*40*	*Female*	*MEŽICA*	*50*
40	*Group3*	*D11*	*60*	*Male*	*MEŽICA*	*50*

41	*X*	*B11*	*126*	*Female*	*MEŽICA*	*50*
42	*Group4*	*D11*	*39*	*Female*	*MEŽICA*	*51*
43	*X*	*C9*	*85*	*Male*	*MEŽICA*	*53*
44	*X*	*D14*	*36*	*Female*	*MEŽICA*	*54*
45	*X*	*D11*	*159*	*Female*	*MEŽICA*	*54*
46	*X*	*I7*	*111*	*Female*	*ČRNA*	*55*
47	*Group4*	*D11*	*160*	*Female*	*MEŽICA*	*55*
48	*X*	*C11*	*107*	*Male*	*MEŽICA*	*56*
49	*Group5*	*C10*	*27*	*Male*	*MEŽICA*	*57*
50	*Group8*	*I10*	*141*	*Male*	*ČRNA*	*57*
51	*Group4*	*C10*	*3*	*Female*	*MEŽICA*	*58*
52	*Group12*	*G12*	*170*	*Female*	*ČRNA*	*58*
53	*Group3*	*D11*	*75*	*Female*	*MEŽICA*	*59*
54	*Group2*	*D11*	*122*	*Male*	*MEŽICA*	*59*
55	*X*	*D11*	*117*	*Male*	*MEŽICA*	*60*
56	*X*	*D11*	*148*	*Male*	*MEŽICA*	*60*
57	*X*	*J10*	*69*	*Male*	*ČRNA*	*62*
58	*Group10*	*H11*	*163*	*Female*	*ČRNA*	*62*
59	*X*	*D13*	*108*	*Male*	*MEŽICA*	*64*
60	*Group3*	*D11*	*161*	*Male*	*MEŽICA*	*64*
61	*Group3*	*D11*	*34*	*Female*	*MEŽICA*	*65*
62	*X*	*C11*	*43*	*Male*	*MEŽICA*	*65*
63	*Group2*	*D11*	*154*	*Female*	*MEŽICA*	*67*
64	*Group9*	*I11*	*41*	*Female*	*ČRNA*	*68*
65	*Group2*	*D11*	*125*	*Female*	*MEŽICA*	*70*
66	*x*	*D11*	*127*	*Male*	*Mežica*	*70*
67	*Group5*	*C10*	*182*	*Female*	*MEŽICA*	*70*
68	*Group4*	*C10*	*79*	*Female*	*MEŽICA*	*74*
69	*X*	*C11*	*32*	*Female*	*MEŽICA*	*75*
70	*X*	*I10*	*169*	*Female*	*ČRNA*	*75*
71	*Group4*	*C10*	*153*	*Male*	*MEŽICA*	*76*
72	*X*	*I10*	*143*	*Male*	*ČRNA*	*77*
73	*Group10*	*H11*	*130*	*Male*	*ČRNA*	*79*
74	*Group10*	*H11*	*8*	*Female*	*ČRNA*	*80*
75	*Group8*	*I10*	*175*	*Male*	*ČRNA*	*80*
76	*Group10*	*H11*	*183*	*Male*	*ČRNA*	*80*
77	*Group10*	*I10*	*173*	*Female*	*ČRNA*	*81*
78	*Group2*	*D11*	*5*	*Male*	*ČRNA*	*82*
79	*Group2*	*D11*	*89*	*Female*	*MEŽICA*	*83*
80	*Group3*	*D11*	*179*	*Female*	*MEŽICA*	*84*
81	*Group4*	*C10*	*15*	*Female*	*MEŽICA*	*85*
82	*Group11*	*H11*	*184*	*Female*	*ČRNA*	*85*
83	*X*	*C11*	*49*	*Male*	*MEŽICA*	*89*
84	*X*	*C11*	*46*	*Male*	*MEŽICA*	*90*
85	*X*	*D13*	*158*	*Female*	*MEŽICA*	*91*

86	X	B11	53	Male	MEŽICA	93
87	Group8	I10	66	Male	ČRNA	94
88	Group2	D11	156	Female	MEŽICA	94
89	X	J9	22	Female	ČRNA	97
90	X	M16	58	Female	ČRNA	100
91	X	H9	103	Male	ČRNA	100
92	X	I10	104	Male	ČRNA	100
93	X	H9	140	Female	ČRNA	102
94	Group12	G12	147	Female	ČRNA	102
95	Group11	H11	14	Male	ČRNA	103
96	X	E13	86	Female	MEŽICA	103
97	X	C11	113	Female	MEŽICA	103
98	Group4	D10	61	Female	MEŽICA	104
99	Group5	C10	123	Male	MEŽICA	104
100	Group8	I10	42	Female	ČRNA	105
101	Group10	I11	112	Male	ČRNA	105
102	X	K13	9	Male	ČRNA	109
103	Group3	D11	100	Female	MEŽICA	109
104	X	J1	136	Male	ČRNA	109
105	Group10	I11	6	Male	ČRNA	110
106	X	C10	20	Male	MEŽICA	110
107	Group10	H11	48	Male	ČRNA	110
108	Group12	G12	52	Female	ČRNA	110
109	Group6	C11	55	Female	MEŽICA	110
110	X	J2	64	Male	ČRNA	110
111	Group4	C11	105	Male	MEŽICA	110
112	Group4	C10	152	Male	MEŽICA	110
113	X	K4	164	Male	ČRNA	110
114	Group3	D11	146	Male	MEŽICA	111
115	Group3	D11	1	Female	MEŽICA	112
116	X	D11	16	Male	MEŽICA	113
117	Group8	I10	81	Male	ČRNA	113
118	Group8	I10	145	Female	ČRNA	113
119	Group10	H11	78	Male	ČRNA	116
120	Group10	I10	142	Male	ČRNA	116
121	Group4	C10	76	Female	MEŽICA	117
122	X	C11	114	Female	MEŽICA	118
123	X	E10	2	Male	MEŽICA	119
124	Group5	C10	17	Male	MEŽICA	122
125	X	J1	93	Male	ČRNA	122
126	X	J14	185	Female	ČRNA	125
127	Group12	H12	135	Female	ČRNA	130
128	Group8	I10	186	Male	ČRNA	130
129	Group3	D11	187	Male	MEŽICA	130
130	Group8	I10	68	Male	ČRNA	131

131	Group8	I10	90	Male	ČRNA	132
132	X	K13	73	Male	ČRNA	133
133	Group1	E11	74	Female	MEŽICA	133
134	Group10	I11	129	Male	ČRNA	133
135	Group4	D11	31	Female	MEŽICA	134
136	X	C9	7	Male	MEŽICA	135
137	Group8	I10	91	Male	ČRNA	135
138	Group5	C10	72	Male	MEŽICA	139
139	Group5	C10	87	Male	MEŽICA	140
140	Group10	I11	30	Male	ČRNA	147
141	X	C11	150	Male	MEŽICA	154
142	Group3	D10	25	Male	MEŽICA	157
143	Group2	D10	38	Male	MEŽICA	157
144	X	K1	92	Female	ČRNA	159
145	Group8	I10	178	Male	ČRNA	160
146	Group1	D12	151	Male	MEŽICA	162
147	X	J9	174	Male	ČRNA	164
148	Group2	D11	24	Female	MEŽICA	167
149	X	I17	94	Female	ČRNA	168
150	X	M16	132	Female	ČRNA	168
151	Group10	I11	96	Female	ČRNA	170
152	Group12	G12	45	Male	ČRNA	172
153	Group10	H11	13	Male	ČRNA	174
154	X	I10	35	Female	ČRNA	180
155	Group8	I10	177	Male	ČRNA	180
156	Group11	H11	188	Female	ČRNA	180
157	Group9	I11	189	Male	ČRNA	180
158	Group4	D10	190	Male	MEŽICA	180
159	X	I7	109	Female	ČRNA	181
160	X	I9	167	Male	ČRNA	184
161	Group4	C11	62	Male	MEŽICA	185
162	Group8	I10	133	Male	ČRNA	186
163	Group10	I10	191	Female	ČRNA	190
164	Group12	G12	10	Male	ČRNA	199
165	Group12	G12	144	Female	ČRNA	200
166	Group10	I10	192	Male	ČRNA	200
167	X	H9	63	Male	ČRNA	204
168	X	I8	80	Male	ČRNA	210
169	Group3	D11	193	Male	MEŽICA	210
170	Group8	I10	83	Female	ČRNA	214
171	X	I11	88	Male	ČRNA	220
172	X	J12	166	Female	ČRNA	220
173	X	I10	57	Female	ČRNA	238
174	Group10	I11	97	Male	ČRNA	238
175	X	H14	51	Male	ČRNA	250

176	*Group5*	*C10*	*194*	*Male*	*MEŽICA*	*250*
177	*X*	*A11*	*195*	*Female*	*MEŽICA*	*250*
178	*Group5*	*C10*	*4*	*Female*	*MEŽICA*	*253*
179	*X*	*I10*	*162*	*Female*	*ČRNA*	*280*
180	*Group3*	*D11*	*120*	*Male*	*MEŽICA*	*282*
181	*Group1*	*E11*	*149*	*Male*	*Mežica*	*297*
182	*Group10*	*I11*	*12*	*Male*	*ČRNA*	*299*
183	*X*	*K4*	*196*	*Male*	*ČRNA*	*300*
184	*Group10*	*I10*	*137*	*Male*	*ČRNA*	*302*
185	*Group10*	*H11*	*98*	*Female*	*ČRNA*	*303*
186	*X*	*E12*	*106*	*Female*	*MEŽICA*	*306*
187	*X*	*J11*	*176*	*Male*	*ČRNA*	*330*
188	*Group12*	*G12*	*99*	*Female*	*ČRNA*	*334*
189	*Group12*	*G12*	*110*	*Male*	*ČRNA*	*337*
190	*X*	*C11*	*28*	*Male*	*MEŽICA*	*340*
191	*Group6*	*C11*	*47*	*Female*	*MEŽICA*	*356*
192	*Group12*	*G12*	*11*	*Male*	*ČRNA*	*364*
193	*X*	*I10*	*44*	*Male*	*ČRNA*	*375*
194	*Group8*	*I10*	*171*	*Female*	*ČRNA*	*420*
195	*X*	*I10*	*77*	*Female*	*ČRNA*	*480*
196	*Group12*	*H12*	*168*	*Female*	*ČRNA*	*500*