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Contents

Scientific review article

Neglected importance of cumulative exposure to phosphorus in drinking water and food products

Gregor **JEREB**[1], Borut **POLJŠAK**[1], Ivan **ERŽEN**[*2]

ABSTRACT

Using a risk assessment approach possible health risks from different chemicals or other environmental stressors could be assessed. The method is widely used for regulatory risk assessment of chemicals. Although the method gives good results in the field of public and environmental health risk assessment, the approach has several flaws and unknowns, since disregarded real exposure scenarios could sometimes also lead to wrong assumptions. Using the risk assessment approach in the case of phosphate additives we will present weather added phosphorus in food and drinking water, presenting some concerns for human health. In recent years in developed countries, according to recent studies, intake of phosphorus and consequently phosphorus serum levels are increasing. Besides naturally present phosphates in food, predominantly pre-processed food and also processed (chemically softened) drinking water is a source of additional phosphate intake. The main reason for drinking water chemical softening is primarily prevention of the equipment; the health effect of such treatment is underestimated and neglected.

Although phosphorus is an essential element, according to latest researches blood vessel calcification and hormonal de-regulation as health effect of high phosphorus concentration are reported. Any kind of increased intake of phosphorus is therefore not needed and in fact it could actually present an additional health threat. Therefore it is necessary that a holistic approach of risk assessment is used in the context of realistic exposure scenarios of simultaneous exposure to cocktail of various pollutants, their degradation products, and inclusion of potential causal links and indirect impacts of evaluated chemicals on health.

According to the presented facts health risk in the case of sodium polyphosphate as drinking water softeners is insufficiently investigated and consequently, the risk might be underestimated.

Key words: risk assessment, polyphosphates, water softening

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INTRODUCTION

Understanding the risk for human health on the field of environmental and public health, mainly due to increasing environmental pollution with different chemicals, is essential to implement appropriate steps to ensure the health of the general population. Often there is not just one "answer" or one "approach" to the question of the risk assessment of substances in the environment [1]. Individuals (and the population) are exposed to various harmful factors in the environment, from which each of them could affect their health differently.

Most of the available information and data on the toxicological endpoints are, unfortunately, available only for one selected chemicals. This lack of information is followed by the traditional approach in risk assessment, which consequently focuses on the assessment of a single chemical, while ignoring the real exposure conditions (co-exposure to a cocktail of different compounds, their interaction and possible synergism or multiplicative effects, the effect of degradation by-products, side effects, and other). This is especially the case in the term of regulatory toxicology and registration of chemicals. Therefore need for a more holistic approach in human health risk assessment of chemicals is needed. Cumulative risk assessment as a tool for analyzing information to examine, characterize and possibly quantify combined threats from multiple environmental stressors [2-4] is one of the answers to these needs. Several studies confirm that exposure to multi-component chemical mixtures pose one common pattern, regardless of the specific chemicals, exposed organism or biological endpoint is observed: toxicity of a chemical "cocktail" is higher than the individual toxic effect of individual observed chemical compound. Same effect can be observed when low, individually non-toxic concentrations of chemicals can result in a sever toxicity, if they co-occur in a mixture. Unfortunately, there are rare studies [5] that take into account the real scenarios and environmentally relevant conditions (low concentrations, lifetime exposure, inclusion of chemical by-products and mixtures). The exposure scenario for intake into the body therefore should consider cumulative exposure from different products and/or media and/or pathways.

According to recent published studies [6-11] phosphorus intake, especially in developed countries, pose a health threat due to high consumption and consequently high serum concentration. High phosphorus serum concentration is related with health threats not only for specific endangers groups like CKD patients [12-14] but also for general population [6-8]. Additionally, the trend of phosphorus intake via everyday diet in developed countries is increasing [8, 15-16], especially due to the high amount of phosphorus additives in pre-processed food.

RISK ASSESSMENT APPROACH

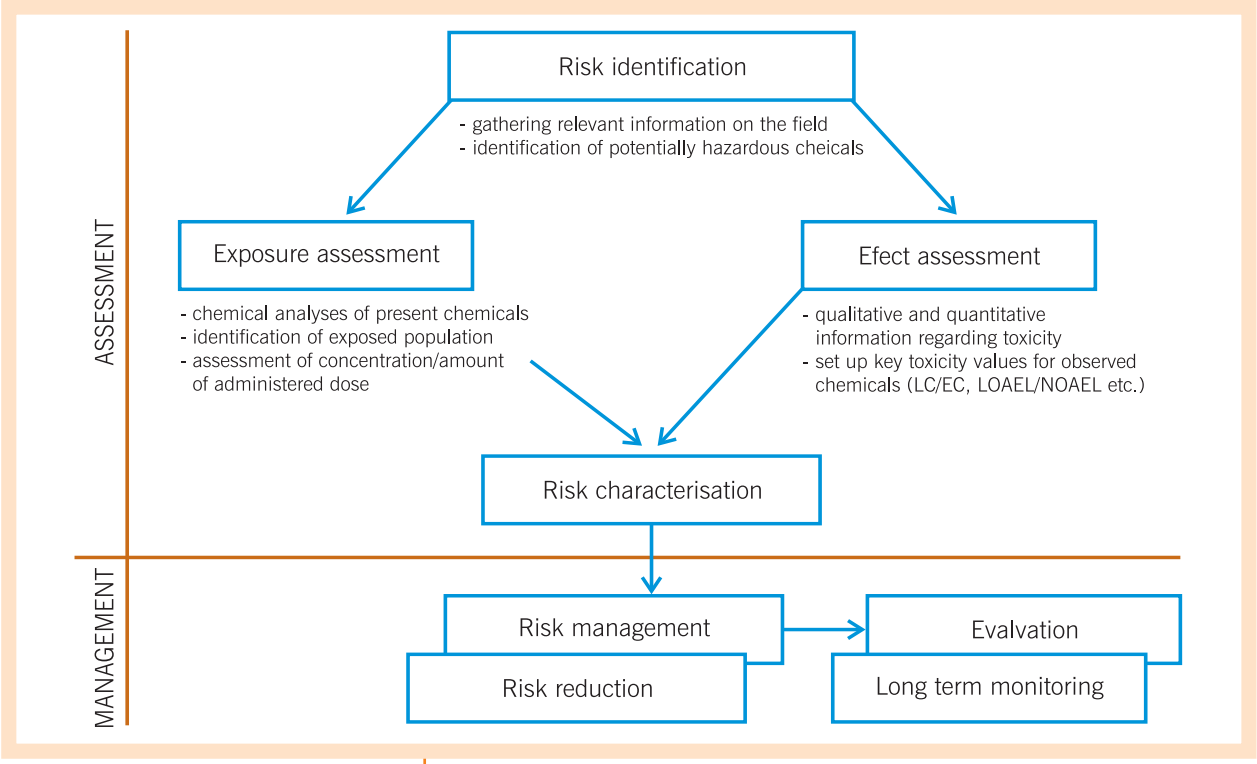
Risk assessment in the content of public health is, to the highest extent possible, a scientific process and represents the method for evaluation and quantification of the probability that harmful effect to individuals or populations from certain chemical or other stressor could occur. The classic approach to risk assessment is based on 4 steps:

1. Risk identification – risk has to be recognized and identified.
2. Hazard assessment and determination of the toxicity (determination of the concentration at which effects are not expected – NOAEL) for a selected chemical.
3. Exposure assessment. Hazard (toxicity) of chemicals does not mean that the deleterious effect on the organism occurred; crucial is contact/absorption in the organism. For exposure assessment data of concentration, route of administration, metabolism, bioavailability and concentration in the target organ is needed.
4. Risk characterization as the final step is done on the basis of the above mentioned steps taking into consideration appropriate safety factors. With such approach the limited values below which no harmful impacts are expected as well as threshold values and acceptable daily intake (ADI) values can be established.

The risk assessment approach should be followed by the risk management, with which we can manage, reduce, reuse, and take other safety measurement, continuous evaluation and correction of risk assessment.

Despite the positive intentions of the classical risk assessment method [1, 17-21] risk assessment approach has several flaws, confronts many unknowns and could sometimes also be based on wrong assumptions.

Figure 1:
Risk assessment approach



Besides neglecting already mentioned real exposure scenarios (especially long-term exposure to low concentration of mixtures) main flaw are related to the uncertainty of the results of toxicological and risk assessment studies (sampling errors, analytic errors, systematic errors, errors due to intra and inter-species extrapolation), influence of different root of exposure on toxic effect, (un)reliability of QSAR (Quantitative Structure Activity Relationship) and SAR (Structure Activity Relationship) models, unreliability of computer models (simplification of complex phenomena in the environment and in the body to linear mathematical models), disregard the impact of hormesis [22-24], multiplicative effects of different chemicals [25], the effects of synergism [26], and antagonism [26], disregarding the impact of degradation by-products, bioaccumulation and bioconcentration by the food chain [27-29], side effects from real case exposure scenarios (by-effects, collateral effects, indirect effect), cumulative effect of daily intake through different sources, and other less significant issues.

In order to show complexity and possible human health threats the case study of added phosphorus in food and drinking water is presented.

CASE STUDY: PHOSPHORUS IN DRINKING WATER

The main reason for drinking water softening is primarily prevention of the equipment such as hot-water boilers, kettles and pipes from limestone formation, especially in case when the source of drinking water is rather hard (carbonate hardness). The main reason for water softening is therefore economical; the health effect of such treatment might be underestimated and neglected. For softening there are several different approaches possible.

Figure 2:
Limestone formation in drinking water pipelines and hot water boilers

Figure 3:
Sodium polyphosphates dosage into the drinking water system

Usually in the domestic distribution network sodium and potassium salts are used, however also softening using sodium polyphosphates is rather common. According to Slovenian legislation [30] adding phosphates in drinking water is not allowed, however it is also not controlled.

Softening is mainly performed on warm, sometimes also in cold drinking water. It is performed on domestic water installation, usually before drinking water enters the boiler for heating.

PHOSPHORUS IN FOOD

Beside intake via drinking water majority of phosphates are ingested via different foods. Phosphorus as food additives is used in several formulations (phosphoric acid (E 338–341; E 343) and polyphosphates (E 450–452)) and is authorized in a large number of food products for several technological purposes. A maximum tolerable daily intake (MTDI) of 70 mg/kg of body weight (bw) of phosphorus was established by several authorities [31-33], however acceptable daily intake (ADI) was not able to be determined because phosphorus (primarily as phosphate) is an essential nutrient and an unavoidable constituent of

food. Based on mentioned expertise limited values of phosphorus additives in different food products are determined [32, 34-35]. However for individual food product these levels are set rather high not including cumulative effect of the daily intake through different products.

In the last few decades therefore phosphorus intakes have risen significantly due to the greater use of phosphates as food additives in different food products [15]. The mean daily phosphorus intake of adults in European countries ranges between 1017 and 1422 mg [16] and between 1030 and 1727 mg for USA population [8], a level well above the current recommendations [8, 36]. Several scientific studies claims increased Phosphorus intake could be linked to several health problems [7, 37]; therefore EU food authorities (EFSA) will re-evaluate phosphates for use as food additives with high priority by the end of the year 2018 [11]. In the context of this re-evaluation all relevant toxicological information will be collected and re-evaluated.

Since phosphorus is an essential microelement and can be found in any cell, it is therefore also "naturally" present in all kinds of food products. However, in animal protein rich food phosphates are present mainly in the form of organic phosphate esters [6] which are slowly hydrolyzed and therefore relatively low absorption (40 – 60 %) is present [38-40]. Plants phytate are less bioavailable and therefore less than 50 % is absorbed [39-41]. On the other hand, for industrial processed food different additives are used, among many of them as polyphosphates or in the form of other inorganic phosphate salts which are almost entirely absorbed in gastrointestinal tract [40]. The same polyphosphate salts are used also in the process of water softening, where again phosphate "additives" are added in to the food (drinking water).

PHOSPHORUS IN EVERY DAY DIET AND HEALTH EFFECT

Phosphorus is an essential element and crucial for cells (and organisms) vital functions. It is widely present in all kinds of food, however according to latest research [6, 8] the amount of phosphorus intake has increased significantly in the last years which lead to unbalance phosphorus homeostasis in the organism. Several researchers [40, 42-46] reported the health effect of high phosphorus concentration among patients with chronic kidney disease, especially higher mortality [43, 45-46]. Recently researchers have stressed the correlation between cardiovascular diseases and high serum phosphorus concentration in the general population [6, 39, 44, 47]. High phosphate serum concentration caused vascular calcification in vitro and in vivo [44, 48].

More and more authors point out that it is necessary that the holistic approach of risk assessment is used in the context of realistic scenarios of simultaneous exposure to whole cocktail of various pollutants, their degradation products, a variety of comprehension and sensitivity of the individual exposed person, especially in the case of specific vulnerable groups and the inclusion of potential causal links and indirect impacts of evaluated chemicals on health. Therefore some additional approaches should be included in health risk assessment for phosphorus addi-

tives, especially holistic view on health effect, such as use of epidemiological data and collateral side effects which phosphorus might have (role of increased phosphorus concentration on hormonal regulation and vascular cell re-programination to osteoblast like cells) instead of assessment only narrow (especially acute) toxicity data.

CONCLUSION

Based on the arguments presented, human health risk assessment for exposure to water softeners (potassium polyphosphates) should be revised; possible interaction between different chemicals (synergism, antagonism, multiplicism) should be included as well as other indirect effects, as well as the effect of cumulative (daily) intake through different sources. Traditionally risk assessments (and also toxicological tests, based on which such risk assessment is made) are made for pure chemicals and ignore all of the above mentioned factors. It is urgent to review such approach in order to determine the interaction between different substances in water and water softeners and, consequently, the phenomenon of multiplication, synergism or antagonism.

As mentioned in the article several authors [8, 15-16] report about the correlation between high phosphorus intake, high serum concentration and health effects, not only among vulnerable groups but also among the general population. Any kind of additional intake of phosphorus is therefore not needed, in fact it could actually present an additional (phosphorus) burden and therefore additional health threat, which is also true in the case of using phosphates as drinking water softeners.

In case of intake of phosphorus the lack of information is crucial. The general public have not adequate knowledge about the potential risk to the cardiovascular system and renal function caused by high phosphorus consumption. Consumers usually also cannot decide for a product with lower phosphorus content due to poor food labelling. The same problems occur in case of drinking water softening, since consumers usually have no idea, that caretaker in their building use such chemical treatment of their drinking water. Usually intake of phosphorus via individual food products is not extremely high and could be negligible compared to the RDA (Recommended Dietary Allowance). In case of drinking water softening recommended values of polyphosphates are rather low compared to RDA for phosphorus daily intake, however it also contributes to the total sum of daily phosphorus consumption, therefore total daily intake of phosphorus could be (and in most cases is) extremely high and above the recommended values.

Based on the mentioned facts we estimate that the health risk in the case of sodium polyphosphate as a drinking water softener is insufficiently investigated and consequently, the risk is probably underestimated.

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Scientific review article

Current and planned activities in the field of water management in Serbia

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ABSTRACT

Integrated water management in Serbia is regulated by the Water Act, which gives a set of measures and activities aimed at maintaining and improving the water regime, providing the required amounts of water of the required quality for different purposes, protecting waters against pollution and protecting against the harmful effects of water. During the past decade, there has been some progress in improving environmental protection in general, but Serbia still bears the consequences of a heritage of environmental degradation. According to current estimates, significant investments in the future will be necessary to solve the problems of wastewater and water supply. Improvements are expected to the existing regulatory and technical framework in this field, as well as clearer structures in competent institutions.

This paper presents the current and planned activities in the field of water management in Serbia.

Key words: water management, wastewater, water supply

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THE CURRENT STATUS OF WATER MANAGEMENT IN SERBIA

Water quality and infrastructure facilities

The Republic of Serbia still faces many challenges in terms of water quality. About 43 % of the surface waters samples in the territory of Vojvodina were categorized as 'very bad' and 'bad' water quality. The maximum concentrations and average annual concentrations of certain heavy metals in most of the lakes and reservoirs for water supply are above the allowable limits.

The water supply network consists of 33,228 km of pipes, and about 79 % of the country's population is connected to the public water supply (92 % in Vojvodina). However, water quality in the water supply system suffers large amounts of pollution, with key sources of pollution including untreated industrial and municipal wastewater, drainage water from agriculture, leachate from landfills, and pollution related to river navigation and operation of power plants. There is also a serious problem in AP of Vojvodina with natural arsenic contamination [1].

Although growing, the percentage of households connected to the public sewer network is still small: only 36 % in Central Serbia and 23 % in Vojvodina. According to Comparative Indicators of Serbian and EU environmental protection service levels, only about 66.7 % of urban wastewater is collected and 11.49 % is treated in Serbia in comparing to the average in EU [2].

Wastewater problems are even more alarming in industry and mining. Only 15 % of the total volume of wastewater from point sources is treated by primary treatment, of which 11 % and 3 % then passes through secondary and tertiary treatment. Only 5 % of industrial wastewater passes all recognized stages of processing (mechanical, biological and chemical). Wastewater facilities exist in only 21 municipalities (out of 219 registered in the Republic of Serbia); even the largest cities in the Republic of Serbia (Belgrade, Nis and Novi Sad) discharge their wastewater without treatment into the recipients. As a result, some locations in the Republic of Serbia (eg, The Grand Backa Canal, Ludosko and Palic lake, the wastewater canal in Pancevo) are extremely polluted with untreated industrial and municipal wastewater. Much of the wastewater infrastructure has not been well maintained in recent decades and there is a substantial maintenance backlog.

Agriculture mainly contributes to the pollution of waters from diffuse sources of pollution, which in the watershed of the Danube gives 70 per cent of total nitrogen, 50 per cent of total phosphorous and 90 per cent of faecal and coliform bacteria. The main agriculture sources of water pollution are the uncontrolled effluents of livestock farms, crops, slaughterhouse wastes and chemical fertilizers. The absence of control of water quality used for irrigation may be a cause of water pollution, although only 2 % of agricultural land is equipped for irrigation. However, it is difficult to estimate to what extent agriculture contributes to water pollution in Serbia, because there is no complete nor effective monitoring of ground water quality [3].

REGULATORY FRAMEWORK

In the field of water protection and management in Serbia, the following regulations are in place: Law on Waters [4], Law on Environmental Protection [5], Law on the Water Regime [6]. In this moment, the Law on Waters is in the revision process. Industrial pollution control and risk management in Serbia are regulated by the Law on Integrated Pollution Prevention and Control [7], which define the conditions and procedures for issuing integrated permits for establishments and activities that may have a negative impact on human health, the environment or material goods; and the types of facility activities, monitoring and other issues of importance in terms of environmental pollution prevention and control.

Transposition of EU directives in the field of water and wastewater is still limited, and can be considered to be in progress. The best results were achieved in the transposition of the Water Framework Directive and Directive on drinking water. Similarly the existing system of flood risk management achieves reasonable compliance with the aims of the Flood Risks Directive, albeit that formal compliance has yet to be fully achieved. Serbia is currently failing to achieve compliance with the main pollution control requirements specified in the Urban Waste Water Treatment Directive and the Nitrates Directive (transposition of these Directives are in progress). This noncompliance results in failure to achieve the environmental objectives of the Water Framework Directive. The Seveso and IED Directives have not yet been fully transposed, although progress has been made. The largest part of the EMAS regulation is still missing.

For the purpose of applying water management law, a set of by-laws were adopted in Serbia during the 2010-2014 period. The release of pollutants from point sources is regulated by the Regulation on emission limit values in waters and deadlines for the achievement thereof [8]. This Regulation sets limit emission values for specific groups or categories of the polluting substances in wastewaters. For the first time, the legislature of the Republic of Serbia has issued all limit values for process wastewaters (by sector) at the facility/unit discharge location, with demands for separating wastewater flows and prohibiting the dilution of these waters in order to achieve the above mentioned values.

The existing facilities, according to this Regulation, will synchronize their emissions with the limit values of pollution emission by 2030/2045 and not later. Legal entities, the entrepreneurs or individuals who discharge their wastewaters in the recipient or the public sewage need to establish deadlines for gradual achievement of pollution limit values. These deadlines should be a part of action plans. For any new facilities being built, the limit values need to be respected immediately. The water quality standards (EQS for water) are regulated in Serbian legislation by the Regulation on limit values of pollutants in surface and ground waters and sediments, and the deadlines for their achievement [9], which transposed the provisions from a number of EU directives regulating this area. The Regulation determines the limit values of polluting substances in the surface and groundwaters, as well as sedi-

ment, along with the deadlines for achieving those values. The limit values are defined for the pollution in the surface waters which present indicators for oxygen, acidity, salinity, nutrient substances, metal organic substances and microorganism indicators content. Additionally, limit values for prioritized and hazard prioritized substances in the surface waters are taken into consideration, defined by the Regulation on limit values of prioritized and hazardous prioritized substances which pollute surface waters and the deadlines for their achievement [10]. This regulation issues quality standards for pollution in surface waters, on which it provides the basis for a 5 class water classification. The classes match ecological status according to the classification given in the regulation which issues the parameters of the ecological and chemical status for surface waters [11].

Defining the limit values of pollution is important for the evaluation of sediment quality, which has also been introduced in the legislature for the first time, in water and environmental management in general. The Regulation issues quality standards for the basic parameters of groundwater quality, providing the basis for monitoring and additional assessment of standards for substances from the minimal list of pollutants of the EU Directive on the protection of groundwater against pollution and deterioration. It issues an obligation to the users of groundwaters, obliging them to perform measurements of the basic (background) level of pollution, ions or natural origin indicators and/or if their presence in the groundwaters is caused by human activity. According to the Regulation, it is prohibited to introduce pollution inside groundwaters if such activity can lead to a worsening of the current state or a deterioration of the current chemical groundwater status respectively, the process being assessed in accordance with the regulations which deal with the water and environmental protection areas. It is forbidden to directly or indirectly release List I and List II pollution into groundwaters.

Significantly, these Regulations present the basis for the new, "combined approach" in water pollution prevention [12]. On the basis of these acts, monitoring of surface and ground water and sediment was carried out in Serbia, which is quite compliant with the requirements of the Water Framework Directive and which is expected to be fully compliant by the end of 2015.

INSTITUTIONAL ARRANGEMENTS

Water management in the territory of the Republic of Serbia shall be exercised through the Ministry of Agriculture and Environment protection and other relevant ministries, autonomous provinces, local government units and public water management companies. The territory of the Republic of Serbia is a unique area divided into seven river water districts. The main water management institution in Serbia is the Directorate for Water of the Ministry of Agriculture and Environment protection. The Directorate initiates policy, drafts laws and has the bulk of policy responsibilities in the sector. Some competencies in the field of water management were passed to the Government of the Vojvodina

Province. The implementation bodies are two public water management companies (PWMC): "Srbija vode" and "Vode Vojvodine". The PWMCs are first and foremost responsible for the maintenance of water control measures, including flood protection, drainage and irrigation. Implementation of these activities are carried out through water management companies. A large number (24) of these companies are in the process of transformation and their final status must be resolved by the end of 2015 (transformation began their transition to PWMC and the parallel process of privatization).

In general terms, the new Water Law clearly defines the responsibilities of the relevant institutions. However, some overlaps in institutional responsibilities do exist, particularly in respect of the control of wastewater discharges. Coordination and cooperation between the institutions is moderately good and continues to improve. Considering that the water issues are divided between several ministries and agencies, cooperation between actors in the future is essential for the achievement of a coordinated approach.

For the Public Utility Companies, the great "institutional" constraint is the capacity of the companies which are responsible for the provision of water services. Water services are provided by Public Utility Companies, which are established on municipal administrative divisions. With the exception of the cities, where there tend to be "water services only" Public Utility Companies of a reasonable size, most (>120) of the 152 Public Utility Companies with responsibility for water also have other responsibilities (including solid waste management, street cleaning...) and are of a relatively small scale.

Many Public Utility Companies do not achieve cost recovery for the water services that they provide, partly as a result of the relatively low tariffs that are charged and partly because of lower than optimal scales of operation and non-specialized operational practices with a lower degree of efficiency [13].

PLANNING DOCUMENTS OF WATER MANAGEMENT

According to [4], planning documents for water management are: the strategy for water management in the territory of the Republic of Serbia, a water management plan, an annual program of water management plans governing the protection against harmful effects of water (plan of flood risk management, and general operating plan for flood protection and plans governing the protection of water, protection plan for water pollution control and monitoring program). These plans are separate but harmonised and entail planning on the basis of both administratively appropriate and hydrologically derived boundaries. The use of administrative boundaries is crucial since all plans must be harmonised with land use plans, which are adopted by local administrations. Of all the above mentioned plans, the general operating plan for flood protection was adopted, but the methodology used during its preparation did not involve risk maps.

THE CHALLENGES OF WATER MANAGEMENT IN SERBIA

The Republic of Serbia needs a big investment program for wastewater management and improvement of drinking water quality. It is estimated that the total cost of meeting the requirements of the EU environmental Acquis will be around €10.6 billion (between now and 2030), the most demanding sectors being water (€ 5.6 billion), waste (€2.8 billion) and industrial pollution (€1.3 billion). An important part of the costs are operational ones, which cannot be covered by international sources and will have to be financed from public budgets, private sources or fees [2].

Future activities include the development of the "Strategy for Water Management in the Republic of Serbia" that will establish a long-term direction for water management. This strategy will be followed by the adoption of water management plans. Several water management plans are to be enacted, including a water management plan for the Danube River Basin. This plan will be prepared by the Ministry of Agriculture and Environmental Protection and the Water Directorate, while the others must be prepared by PWMC "Srbija vode" and "Vode Vojvodine" for river basin districts within their jurisdiction. The plans to implement the Directive on Urban Waste Water Treatment and the Nitrates Directive, as well as plan for water pollution and plan for flood risk management are expected also. The importance of Plan to protect water from pollution should be highlighted, because of the implementation of a Regulation on ELVs and the preservation of water quality generally.

According to [14], the instruments of importance to regional cooperation, such as the support of the IPA cross-border and transnational cooperation programs, will be provided for those activities in which there are obvious advantages to a regional approach. In the context of regional priorities in which the IPA are engaged in the environmental sector, the Republic of Serbia supports those relating to the preparation and implementation of investment projects under the Investment Fund for the Western Balkans. In order to complement national efforts, the emphasis is on infrastructure investments related to water management and improving water quality with regional impact on the environment.

The Republic of Serbia is able to finance additional regional projects in the field of climate change and the inclusion of climate change considerations within different projects through the "window" for climate change. Areas for intervention within the cross-border and transnational cooperation program include support for the improvement of water supply; reducing leakages and losses and improved wastewater treatment. IPA cross-border cooperation can also be used to support the development of cross-border environmental protection plans, risk assessments and plans for monitoring in selected transboundary areas. The activities proposed for the implementation of Pillar II Strategy EU Danube Region in the field of environmental protection will directly contribute to the objectives of Europe 2020. These activities relate to the objectives of addressing the challenges of climate change, developing sustainable use of resources (which includes water), as well as ensuring the quality of life, which requires a regional approach to water management.

The overall strategy of Serbia in the water sector is to continue to determine its own priorities, principally those that contribute to socio-economic welfare, whilst continuing to honour its commitments under international agreements and make continued progress towards compliance with EU legislation. In the long-term the key objectives can be summarised as: fully compliant and affordable centralised public water supply to at least 93 % of the inhabitants of Serbia; provision of affordable wastewater collection and appropriate treatment to all agglomerations over 2,000 population equivalent. The achievement of these objectives entails a number of choices in respect of specific aspects of approximation for the short and medium term.

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Original scientific article

Consumer's attitude and manipulation of raw milk from milk vending machines

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ABSTRACT

Despite the high nutrition level of raw milk, consumption of it poses a health risk for consumers, especially high-risk populations. The aim of this survey was to determine consumer's attitude towards raw milk from milk vending machines (n = 305) and to investigate their habits in manipulation of raw milk from the time of purchase until consumption (n = 74). Survey results show that the majority of consumers purchases milk once a week, using their own glass bottle and consume the milk in approximately two days. In most cases, consumers transport milk to their homes in less than 15 minutes without the use of a cooler bag. The manner of bottle cleaning technique depends on bottle material. Almost half of the consumers boil milk before consumption, which is associated with their attitude towards the safety of raw milk consumption. Most of them are satisfied with the level of hygiene on milk vending machines. The majority of consumers is unfamiliar with health risks of raw milk consumption and correct usage.

Key words: raw milk, milk vending machines, consumer's attitude, manipulation, food safety

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INTRODUCTION

Milk represents one of the basic foods in the human nutrition for all age groups, for it contains a high level of nutrients with wide spectrum of proteins, minerals, calcium, phosphate, essential amino acids, especially lysine and vitamins B2 and B12 [1-3]. It is scientifically proven that milk and dairy products positively affect development and function of the gastrointestinal tract and the immune system, growth and function of microbiota, which also includes probiotic function [4]. High level of calcium has an important role in development, firmness and solidity of bones in childhood and adolescence and in maintenance of bone mass in adulthood [1, 4]. In combination with other nutrients in milk calcium also modulates adipocyte metabolism of lipids [4]. Lactose, as the main carbohydrate in milk, has a low glycemic index and at the same time speeds up the absorption of calcium and magnesium [3].

Raw milk is a high quality food with a high nutrition level [1-3], but it is important to be mindful of the health risks that consumption of raw milk poses, of which World Health organization (WHO) and other government organizations also alert [2, 5]. Consumption of raw milk poses a risk for infection with *Listeria monocytogenes*, *Coxiella burnetii* (Q fever), *Campylobacter jejuni* and *coli*, *Salmonella* spp., enteropathogenic *Escherichia coli (EPEC)* and enterotoxigenic *(ETEC) E. coli*, *Yersinia enterocolitica, Staphylococcus aureus,* etc [3, 6]. Pathogenic microorganisms can cause health problems to any consumer, but especially to young children, pregnant women, elderly, chronically ill and immunodeficient people. Pregnant women are an especially vulnerable population, for infection with Lysteria monocytogenes, which can be found in raw milk, poses a danger to the embryo, fetus and infant (abortion, stillbirth and neonatal death) [6, 7].

It is recommended to boil the milk to kill pathogenic microorganisms, particularly if the milk is being consumed by a member of a high risk health group [7]. European regulative defines the criteria for hygiene of raw milk: plate count number at 30 °C ≤ 100.000 cfu/mL and somatic cell count ≤ 400.000 cfu/mL [8].

Raw milk vending machine is an automatic dispensing system and is usually positioned in a place with high frequency of passers-by. Vending machines have been used in Slovenia since 2010. They are operated by farmers who are also producers of the milk being sold. Normally a vending machine is being refilled once a day. Hygienic suitability of the milk and of the vending machine are responsibilities of the owner. The temperature of raw milk must be regulated and must not exceed 4 °C, the temperature must be displayed on a vending machine for consumer's knowledge. Raw milk can be poured in consumer's own bottles or in the bottles purchased in a vending machine located in its vicinity [2].

Past microbiological analyses of raw milk from vending machines showed that milk was not always hygienically adequate, as shown by increased plate count number [9-13] and increased somatic cell count [13, 14]. Consumption of raw milk from a vending machine also poses a chemical hazard [15]. These deviations show a potential hygienic risk of raw milk consumption [9, 13].

To ensure a safe consumption it is important to implement a good hygiene, farming and manufacturing practice, elements of which are presented and defined in European regulations [8, 16]. The elements of good practices are: hygiene-technic requirements (holdings, premises and equipment), maintaining the hygiene level during milking, collection and transportation of milk, handling of waste milk, control of pests and staff hygiene, etc. [8, 16]. For a better detection and prevention of hazards an owner must provide a control system over critical control points with the HACCP system (Hazard analysis and critical control point) [16, 17]. Health unsuitability of raw milk is usually a consequence of poor hygiene circumstances and human sloth [18-21]. For example, it is of a great importance to strictly maintain the cold chain of raw milk before the consumption as this is the only way to prevent excessive bacterial growth; the number of microorganisms at 21 °C doubles in one hour [22].

In Europe the highest part (39.7 %) of food poisoning occurs in households. Most foodborne illnesses caused by raw milk consumption are due to infections with *Campylobacter* spp. In 2012 as high as 20.0 % of all *Campylobacter* infections were caused by milk consumption, one year before, in 2011 quota of this infections was assessed as 13.5 % [23]. Campylobacteriosis was the zoonosis with the highest number of human confirmed cases in 2012 [23].

The aim of this study was to investigate consumer's attitude towards raw milk from milk vending machines and their habits and handling in milk manipulation from the time of purchase until usage.

METHODS

This cross-sectional study about consumer's attitude and manipulation of raw milk from milk vending machines was conducted from November 2013 to February 2015 in Municipality Ljubljana – the capital of the Republic of Slovenia.

Data was collected on weekday mornings and afternoons. There were 305 random passers-by and 74 random consumers of milk from milk vending machines included in the survey. Gender and age of respondents were controlled to ensure a balanced randomized sample. All surveys were conducted by two trained interviewers.

This study is divided into two sections: (1) consumer's attitude towards raw milk from milk vending machines and (2) consumer's manipulation of raw milk from milk vending machines.

Questionnaire design

Questions were both open- and close-ended. The questionnaire consisted of demographic questions (gender, age and place of residence), seven questions about consumer's attitude towards raw milk from milk vending machines and 20 questions about manipulation of raw milk from milk vending machines.

The first part of questionnaire (demographic characteristics and consumer's attitude towards raw milk from milk vending machines) was completed by both those who purchase and those who do not purchase raw milk from milk vending machines. The second part of questionnaire (manipulation of raw milk from milk vending machines) was completed only by those who purchase raw milk from milk vending machines. The first part of questionnaire took approximately three minutes to complete and the second part took between five to ten minutes to complete.

The questionnaire was pilot tested by 20 participants in October 2013 to confirm question clarity, identify response options, and gauge likely interview duration. The questionnaire was revised on the basis of pretest results and other recommendations.

Data analysis

Quantitative analysis

The questionnaire responses were statistically analysed with SPSS Statistics 21.0 (IBM ®). Mean responses with standard deviation and percentages of responses in each category were calculated and presented in tabular form. To examine the relationship between variables, cross-tabulation and χ^2 test were used.

Qualitative analysis

Qualitative analysis was used to evaluate the responses of three open-ended questions. Consumer's responses were displayed as codes, which were categorized in categories [24].

For analysing the open questions "Why do you decide or not decide for the purchase of raw milk from a vending milk machine?" and "What should be changed in the offer of milk vending machine?" we used the description method based on qualitative content analysis [25, 26], which is based upon grounded theory [27]. This methodology helped us determine respondent's motivation for the purchase of raw milk from vending milk machines. Validity of the interpretation is justified by the expert triangulation. Three researches of different expert pre-knowledge and research experiences were included in the interpretation. The basis for the qualitative content analysis represented the transcripts of the answers to the mentioned question. The answers were suitably marked and a numbers of individual questionnaires were added to the research code (CFS). The content analysis was started by assigning the codes to the major topics. The codes were assigned to individual statements or parts of the statements accordingly to repeated reading of the answers. Codes were grouped in thematic categories. The categories and the codes had been upgraded until the majority of the respondent's statements could be organized.

RESULTS AND DISCUSSION

Consumers attitude towards raw milk from milk vending machines

There were 305 completed questionnaires. Demographic features of survey participants are presented in **Table 1**. The percentage of genders was almost equal; the largest group of participants were 26 to 55 years old inhabitants of Ljubljana (27.2 %).

Questioning took place in shopping centres (54.1 %) and on the streets (45.9 %) of Ljubljana. Raw milk from milk vending machines was reported by 21.3 % of respondents, 9.2 % buy milk directly from fairy farms and 69.5 % purchase milk in supermarkets. Before the milk vending machine marketing, in year 2006, the percentage of people who purchased milk in the supermarkets was higher (85.4 %) [28]. The proportion of people who purchase milk on vending machines did not vary regarding the location of questioning. Data on demographic characteristics of milk vending machine users and non-users are presented in **Table 2**.

Table 1: Demographic characteristics of respondents

Demographic characteristics (n^a = 305)	n^a	Proportion (%)
Gender		
Male	148	48.5
Female	157	51.5
Age group		
≤ 25 years	78	25.6
26-55 years	142	46.6
≥ 56 years	85	27.9
Place of residence		
City (Ljubljana)	172	56.4
Ljubljana suburbs	61	20.0
Other cities	72	23.6
Location of questioning		
Shopping centre	165	54.1
Streets, squares	140	45.9

[a] Number of respondents

Table 2: Demographic characteristics of respondents, regarding if they purchase raw milk on milk vending machines or not

Demographic characteristics (n^a = 305)	Purchase raw milk (n^a = 65)		Do not purchase raw milk (n^a = 240)	
	n^a	%	n^a	%
Gender				
Male	31	10.2	117	38.4
Female	34	11.1	123	40.3
Age group				
≤ 25 years	14	4.6	64	21.0
26-55 years	37	12.1	105	34.4
≥ 56 years	14	4.6	71	23.3
Place of residence				
City (Ljubljana)	43	14.1	129	42.3
Ljubljana suburbs	11	3.6	50	16.4
Other cities	11	3.6	61	20.0
Location of questioning				
Shopping centre	32	10.5	133	43.6
Ljubljana streets	33	10.8	107	35.1

[a] Number of respondents

The majority of consumers who purchase milk on vending machines do so because they think that this milk is superior in quality (43.3 %) and healthier (20.0 %). The most prevalent reasons for not using milk vending machines are little or no milk consumption (27.8 %) and because milk is easier to purchase in supermarkets (16.1 %).

Results of qualitative analysis

Consumers mostly purchase raw milk on milk vending machines because they are of an opinion that this milk is superior in quality and healthier, they trust in the milk origin, what they also like is that the raw milk vending machines are easily accessible and available at all times. Beside other, less represented causes consumers prefer this milk for its unprocessed characteristics. On the other hand, survey participants, who do not consume raw milk from milk vending machines, do not decide for this purchase because they do not like its organoleptic properties, raw milk duration, price, milk processing and inaccessibility of milk vending machines.

Others consume milk of a different origin and distrust in the milk itself. Further it is important to acknowledge that some survey participants, due to their health or their particulates in nutrition, consume little or no milk in general.

Consumers habits regarding raw milk from milk vending machines

A total of 74 questionnaires were obtained. Demographic characteristics of participants are listed in **Table 3**.

More than a quarter of all analysed consumers purchase milk on vending machines once a week (28.4 %) or 3 to 5 times a week (27.0 %), which is comparable to study of Golčman [29], where it was discovered that 22.0 % of consumers purchase milk from vending machines once a week. Almost two thirds (60.8 %) of consumers buy 1 L of milk at a single purchase and one quarter (24.3 %) buys 2 L. Milk is being used in two days by 36.5 % and in three days by 31.1 % of consumers.

Table 3: Demographic characteristics of consumers that purchases raw milk on milk vending machines

Demographic characteristics (n^a = 74)	n^a	%
Gender		
Male	36	48.6
Female	38	51.4
Age group		
≤25 years	13	17.6
26-55 years	43	58.1
≥56 years	18	24.3
Place of residence		
City (Ljubljana)	51	68.9
Ljubljana suburbs	12	16.2
Other cities	11	14.9

[a] Number of respondents

The great majority (90.5 %) of consumers never check the temperature displayed on the vending machine and 91.9 % never use a cooler bag for transport. The reported time of transportation of raw milk from vending machine to respondent's homes is less than 15 minutes in 78.4 % of participants, 15 to 30 minutes in 14.9 % and over 30 minutes in 5.5 %; even among the latter group only 4.1 % use a cooler bag. The most probable reason why consumers do not use a cooler bag was already discovered in the study Jevšnik [28], where they found that 33.0 % of consumers think the use of a cooler is not needed and 51.7 % had never thought of a use of a cooler bag. Maintenance of cold chain during the transportation is important, for the temperature significantly influences generic time of microorganisms [6]. 91.9 % of consumers store milk in refrigerator, but in the study of Jevšnik [28] was concluded that more than two thirds of consumers (67.8 %) never check the temperature in the refrigerator and that the correct storing temperature (1-5 °C) is known to only 55.1 % of consumers.

Almost half of consumers (48.7 %) boil raw milk from a vending machine before consumption, 12.2 % boil it only occasionally. Organizations Food and Drug Administration, Centre for Disease Control and Prevention and National Institute of Public Health alert, that consumption of raw milk poses health risks [2], nevertheless 39.2 % of consumers still do not boil the milk before consumption. Boiling of milk is similar in all age groups (53.6 % of consumers aged 25 or younger, 50.0 % aged between 26 and 55 and 44.4 % aged 56 or more. On average raw milk from a vending machine is consumed by 3 people from one household (SD = 1,52). Milk is mostly used for preparation of drinks (66.0 %) or for cooking and baking (59.5 %). More detailed data of consumer's manipulation of raw milk from vending machines is displayed in **Table 4**.

Table 4: Habits of consumers of raw milk on milk vending machines

Query	Response	%
How often do you buy raw milk from milk vending machines? (n[a] = 74)	Every day	4.1
	3-5 times a week	27.0
	Once a week	28.4
	Less than once a week	13.5
	Once a month	17.6
	10 times or less in a year	5.4
	Till now I bought it 5 times or less	4.1
How much milk do you buy at a single purchase? (n[a] = 74)	0.1 L	4.1
	0.2 L	0.0
	0.5 L	4.1
	1 L	60.8
	2 L	24.3
	More than 2 L	6.8
Approximately how long does it take you to use milk from milk vending machines? (n[a] = 74)	1 day	13.5
	2 days	36.5
	3 days	31.1
	More than 3 days	18.9
Do you check the temperature of raw milk, which is displayed on milk vending machine? (n[a] = 74)	Yes	8.1
	No	90.5
	Occasionally	1.4
How much time does it take you to transport the milk home? (n[a] = 73)	≤15 minutes	78.4
	15-30 minutes	14.9
	30-60 minutes	1.4
	60-120 minutes	2.7
	≥120 minutes	1.4
Do you use a cooler bag for transportation of milk? (n[a] = 73)	Always	0.0
	Never	91.9
	Only in the summer	2.7
	Occasionally	4.1
Where/how do you keep the milk at home? (n[a] = 74)	In the refrigerator	91.9
	At standard temperature	7.0
	In the cellar	1.6
Do you boil milk before usage? (n[a] = 74)	Yes	48.7
	No	39.2
	Occasionally	12.2
For what purposes do you use raw milk from milk vending machine? (n[a] = 74)	Preparation of drinks	66.2
	Consumption of raw milk	31.1
	For cooking/baking	59.5
	For making fermented milk products	31.1

[a] Number of respondents

When purchasing raw milk from vending machines 70.3 % of consumers use their own bottle, and 29.7 % purchase bottles every time from a vending machines. At the beginning of raw milk from vending machines marketing, in 2010, half of the consumers used their bottle other half purchased the bottles on vending machines [29]. 52.7 % of consumers use glass bottles and 44.6 % plastic bottles. Plastic bottles are replaced at least once a month by 47.1 % of consumers and glass bottles by 19.3 %. Plastic bottle has never been changed by 5.9 % of consumers and glass bottle by 30.8 %. Consumers with their own glass bottle (37.1 %) and those with their own plastic bottle (45.0 %) mostly clean it with hot water and dish cleaner. Cleaning brush is being used more by those with glass bottle (37.2 %) than those with plastic bottle (10.0 %). The correct way to clean a bottle is to firstly flush it with warm water, secondly wash it with hot water and dish cleaner and in the end it should be again flushed with hot water. It is important to avoid hot water during first flush to prevent denaturation of milk proteins, because with the beginning colder flush we remove the most of the proteins of bottle wall without their denaturation, which can make the removal of proteins harder [30, 31]. The correct cleaning technique is being practiced by only 5.0 % of consumers who use plastic bottles and by none from the glass bottle users. The incorrect cleaning technique can enlarge the possibility of biofilm formation on the inside surface of bottles, which may represent higher health risks [32, 33].

More detailed data of the bottle type that is being used, frequency of bottle exchange and cleaning techniques is being displayed in **Table 5**.

While 73.0 % of participants are confident that raw milk consumption does not pose any risk for health, 17.4 % of participants think that consumption of raw milk does pose health risks and 9.6 % have no opinion on the subject. Opinion on safety of raw milk consumption between different age groups of participants shows that all aged 25 or younger, 69.8 % aged between 26 and 55 and 61.1 % aged 56 or older think that raw milk consumption do not pose a health risk. Overall milk is usually boiled by 75.0 % of those ($n = 13$) who believe raw milk is a potential hazard for health and by 43.4 % of those ($n = 54$) who think it is not. Boiling of raw milk is associated with the opinion that consumption of raw milk poses a health risk ($\chi^2 = 6.77$; $p = 0.034$).

Even though people trust in health and hygienic suitability of raw milk from a vending machine, previous studies have shown that raw milk from a vending machine is not always suitable for consumption. In the spring suitable plate count number has had 76.4 % [9] and 88.0 % of samples [10] and only 35.4 % of samples in the summer [9]. In the summer-autumn time suitable plate count number has had 60.0 % of samples [10], in the autumn 70.6 % [9] and in the winter 51.7 % of samples [11]. Hygienic suitability differs from one vending machine to another. In the previous study of Galičič et al. (2014) it was reported that 82.4 % measured central temperatures of raw milk exceeded 4 °C [9]. Slovene Consumer's Association has had also hygienically unsuitable results (54.5 %) regarding measured central temperatures of raw

Table 5: Respondent's answers regarding bottle type, frequency of bottle exchange and cleaning technique

Query	Response	%
What kind of packing do you use for milk? (n[a] = 74)	My own packing	70.3
	Packing I purchase at the vending machine	29.7
From what kind of material is your packing that you use? (n[a] = 74)	Plastic	44.6
	Glass	52.7
	Plastic or glass	2.7
How often do you change your own bottle? (n[a] = 26)	Once a week	0.0
	Twice a month	3.9
	Once a month	15.4
	Once on 2 months	7.7
	On 2-6 months	11.5
	Once every 6 months	11.5
	Once a year	7.7
	I don't change my bottle	30.8
	Other	11.5
How often do you change your own plastic bottle? (n[a] =17)	Once a week	11.8
	Twice a month	17.6
	Once a month	17.6
	Once on 2 months	11.8
	On 2-6 months	11.8
	Once every 6 months	11.8
	Once a year	11.8
	I don't change my bottle	5.8
	Other	0.0
How do you clean your own bottle? (n[a] = 35)	Flushing with cold or warm water	2.9
	Flushing with hot water	2.9
	Washing with hot water and dish cleaner	37.1
	Washing with the brush, hot water and dish cleaner	22.9
	Washing with the brush and hot water	14.2
	Washing in the dishwasher	17.1
	First I flush it with warm water, than I wash it with hot water and dish cleaner and in the end I flush it again with hot water	2.9
How do you clean your own plastic bottle? (n[a] = 19)	Flushing with cold or warm water	5.3
	Flushing with hot water	21.1
	Washing with hot water and dish cleaner	47.2
	Washing with the brush, hot water and dish cleaner	5.3
	Washing with the brush and hot water	0.0
	Washing in the dishwasher	5.3
	First I flush it with warm water, than I wash it with hot water and dish cleaner and in the end I flush it again with hot water	5.3
	Other	10.5

[a] Number of respondents

milk [12]. It is proven that ambient air temperature and seasonality do not have an impact on hygienic suitability of raw milk from milk vending machines [9, 34]. Somatic cell count has also been found to be increased in raw milk [13, 14]. Residues of detergents in rising solutions of milk vending machines, which are being used for cleaning the chamber and the tube form which milk flows, were 21.7 % of them were found also the residues of detergents and disinfectants [15].

The majority of respondents (98.6 %) have never purchased spoiled raw milk from milk vending machine and the majority (97.3 %) is also satisfied with the level of vending machine hygiene. Mean assessment (Likert scale from 1 (poor hygiene level) to 5 (the highest hygiene level)) of hygiene of a vending machine filling chamber is 4.4 (SD = 0.7). Frece [14] came to the same conclusion in her study, where they discovered that appropriate level of hygiene of milk vending machines filling chambers has only 47.1 % of milk vending machines and 70.6 % of trash bins.

To 90.5 % of consumers the instructions for use of the milk vending machine seemed clear, while for 1.4 % they were not clear enough. 74.3 % of consumers were satisfied with the milk vending machine's offer. More detailed data on the subject of consumer's attitude towards raw milk from vending machines is presented in **Table 6**.

Table 6: Respondent's attitude towards raw milk from vending machines

Query	Response	n[a]	%
Do you think that consumption of raw milk poses a health risk? (n[a] = 74)	Yes	13	17.6
	No	54	72.0
	Does not know	7	9.4
Have you ever purchased rotten raw milk from milk vending machine? (n[a] = 72)	Yes	1	1.4
	No	71	98.6
Do you think the instructions for usage of the vending machine are clear enough? (n[a] = 74)	Yes	67	90.5
	No	1	1.4
	I have never read them	3	4.0
	I do not remember them	1	1.4
	I do not have an opinion	2	2.7
Are you satisfied with the level of hygiene of milk vending machines? (n[a] = 73)	Yes	71	97.2
	No	1	1.4
	Other	1	1.4
Do you think a change of offer available in the milk vending machines would be suitable? (n[a] = 74)	Yes	19	25.7
	No	55	74.3

[a] Number of respondents

Other consumers wish for additional products (ecological production, soured milk, quark, cheese, yoghurt, chocolate milk and other milk products), better accessibility, different milk origin, traceability throughout the process, higher level of hygiene of vending machines, price changes, additional instructions and information and improved technology of vending machines (calibration of milk dosage, lower position of the filling chamber).

CONCLUSION

With this cross-sectional study we have determined consumer's attitude towards the safety of raw milk consumption and have terminated consumer's manipulation with raw milk from the moment of purchase to the time of consumption at home. With this the purpose of this study has been achieved.

In general, consumers purchase raw milk from milk vending machines because they believe that this milk is of a superior quality and is healthier than processed milk available in the stores. Others do not decide for the purchase of raw milk from milk vending machines because they chiefly consume little or no milk. Consumer's manipulation of raw milk from vending machines shows consideration of the most basic food-safety principles, which concretely cover raw milk handling. On the other hand, concomitant elements are given less, most of the time not enough attention. When handling with raw milk from vending machines consumers consider the most basic recommendations of health organisations and also the fact that raw milk is a perishable food, it is especially well considered quick transport timing and correct milk storage, still not enough consumers boil raw milk before consumption. Consumers do not always act in accordance with good housekeeping practice [28]. In our research we found that they rarely use a cooler bag while raw milk transportation and they do not clean bottles efficiently.

In future, it would be important to educate consumers who purchase raw milk from vending machines of the correct way of manipulation of raw milk, as only a conscious consumer can become an active participant in food safety chain [28].

LIMITATIONS OF THE STUDY

For further researches and more representative results the location of this study should be extended to other regions. Closed-ended questions are another potential drawback because of the limited options for responses, this might have influenced some respondents to choose an answer they otherwise would not and as such does not necessarily represent a clear image of an average consumer of raw milk from vending machine. Regardless this design was essential for the statistical analyses, furthermore we have accomplished to wholly present consumer's attitude towards raw milk consumption and have as first in Slovenia analysed and presented consumers handling with raw milk from milk vending machines from the time of purchase to the time of consumption.

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Original scientific article

Methodology for defining the effects of outdoor air pollution on children's health at the population level – a systematic review

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ABSTRACT

Outdoor air pollution is an important determinant of health. Children are one of the most sensitive population groups due to their yet underdeveloped respiratory system. Methodology for linkage environmental and health data at population level had been initiated by the World Health Organization about twenty years ago. The aim of our study is an overview of methods with which the effects of outdoor air pollution on children's health have been investigated at the population level. Literature overview was made systematically. Health effects of outdoor air pollution at the population level were firstly investigated after 1990. Simultaneously with the most common outdoor air pollutants (NO_2, SO_2, O_3, PM_{10} and CO) monitoring of health effects was measured. Poisson regression analysis was the most frequently used method of ecological time-series studies and spatial studies. Exposure misclassification was in this research field the most common limitation of ecological studies. This study supports the need of future research on outdoor air pollution's effects on the at population level from an engineering and public health view.

Key words: outdoor air pollution, children health, time-series study, spatial study, methodology

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INTRODUCTION

In Europe in 2012 482,000 people died of outdoor air pollution-related causes. Outdoor air pollution occurs when the concentration of pollutants in air exceeds the level which has adverse health effects or adverse effects on environment [1]. In average, people daily breathe between 10 to 20 m^3 of air, depending on their physical constitution and physical activity [2, 3]. Excessive outdoor air pollution represents a health hazard for population [4].

One of the first evidences of adverse health effects of air pollution at the population level is so called the Great Smog during December 1952 in London. Thousands of people have died because of the high levels of sulphur dioxide (SO_2) and particles of different diameter present in outdoor air. The main reason for the incident was associated with temperature inversion which had trapped (and formed a thick layer of) SO_2 and smoke. Since then many epidemiological studies have confirmed that short-term exposure to outdoor air pollution is connected with morbidity and mortality [5].

There are about 200 different pollutants present in urban outdoor air. The most common outdoor air pollutants are particulate matter of different diameters, ozone (O_3), nitrogen oxides (NO_x), sulphur dioxide (SO_2), carbon monoxide (CO), heavy metals, volatile organic compounds and pesticides [6].

Both short- and long-term exposure is associated with high risk of respiratory and heart diseases, stroke and lung cancer. The exposure has stronger effect on children, elderly, poor and ill [1]. Outdoor air pollution is strongly associated with chronic respiratory diseases which often appear in childhood. Asthma and other lower respiratory system diseases are in children strongly associated with exposure to NO_2, SO_2 and PM_{10} [7-9]. Outdoor air pollution is also associated with daily visit of primary care health system and hospital admissions [10-14].

The association between outdoor air pollution and its effects on human health can be examined at the individual or at the population level [15, 16]. Studies at the population level were firstly initiated by the World Health Organization (WHO) about twenty years ago, when the usage of so called "linkage methods for environment and health analysis" was recommended [17, 18] as a useful tool in determining the association between environmental pollution and its health effects. Time-trend studies are studies that compare disease rates over time in one population (Morgenstern, 1982; Morgenstern and Thomas, 1993). Spatial studies, usually referred to as multi-group studies, are studies that compare disease rates among many spatial units during the same period [19, 20].

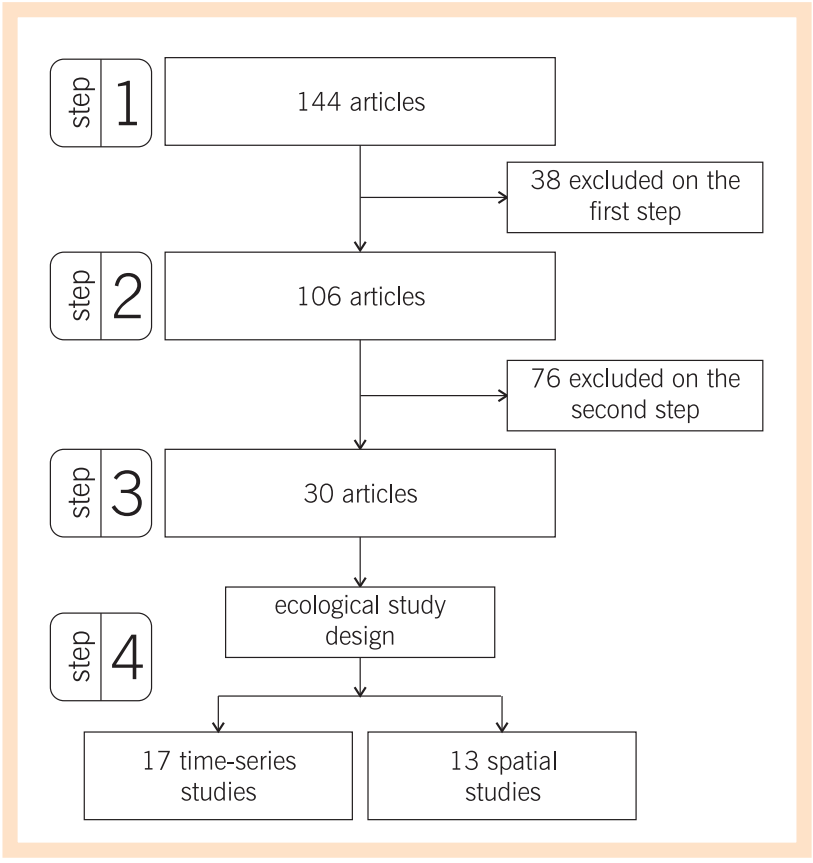
The aim of our systematic review is an overview of methods of epidemiological studies with which the effects of outdoor air pollution on children's health have been investigated at the population level. The specific goal of this review is the presentation of the progress in new findings of the effects of outdoor air pollution on children's health. For

this purpose the overview of scientific articles on this field was made, with the emphasis on methods of defining the association between pollution and effect health effects at population level with the view of the opportunity for future research.

METHODS

Overview of articles on the topic of outdoor air pollution's effects on children's health was arranged chronologically in database PubMed Central. Overview of articles was made in four steps. In each next step new inclusion and exclusion criteria were added.

In the first step we included original and review scientific articles in English language with free full text available and with the publication date between January 1st 1977 (publication year of first article on this topic) and October 17th 2015. In the second step we have excluded the articles which did not include the topic of outdoor air pollutions effect's on children's health. In the third step articles were divided into the groups depending on study design. In the last, fourth step epidemiological ecological studies were analysed precisely. Observed health outcome, exposure to outdoor air pollution and methods of defining the association between outdoor air pollution and health effects at the population level are displayed in tabular form. In databases epidemiological ecological studies that were made in Slovenian area were looked for, the results of this search is also displayed in tabular form (Figure 1).

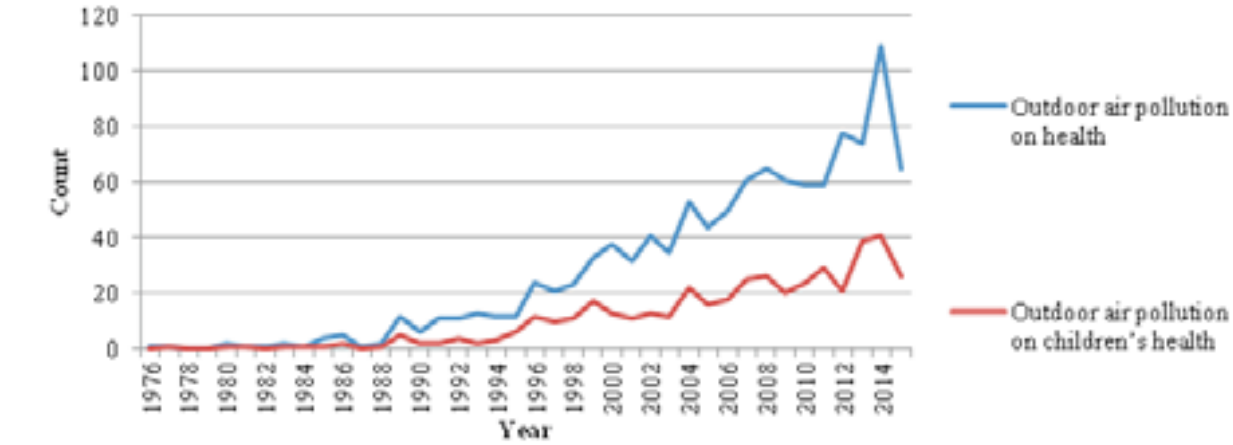


Picture 1:
Flow diagram of the search process

RESULTS

The progress in new findings of the effects of outdoor air pollution on children's health

The first epidemiological article on the health effects of outdoor air pollution was published in 1976. Already in the next year, in 1977, the same effects were investigated on children. Picture 2 shows number of original scientific articles (epidemiological and toxicological study design) and review articles.



Picture 2:
Publication trends for the effect of outdoor air pollution on health (blue) and the effect of outdoor air pollution on children's health (red) at the population level from January 1st 1976 to October 17th 2015 [21].

Methods of association between outdoor air pollution and children's health effects at the population level

In our systematic review we focused on ecological study designs; time-series studies and spatial studies. Overview of time-series studies is displayed in Table 1 and spatial studies in Table 2.

Table 1: Overview of ecological time-series studies at the population level included in systematical analysis

Research	Period of observation	Observed health outcome	Exposure to outdoor air pollution		Meteorolo-gical parameters	Methods
			Pollutants	Lag [day]		
Buchdahl et al., 1996 [22]	Mar. 1992 – Feb. 1993	Daily incidence of acute wheezy episodes	Daily av. conc.: O_3, SO_2, NO_2	1, 2, 3, 4, 5, 6, 7	T, wind speed	Poisson regression analysis
Morgan et al., 1998 [10]	Jan. 1990 – Dec. 1994	Daily hospital admission of asthma	Particulates$_{0.01\ to\ 2.00\mu m}$ (daily av. conc.,), NO_2 (daily av. conc., daily max. 1-hr conc.); O_3 (daily max. 1-hour)	0, 1, 2	T (mean, dew point)	Poisson regression analysis
Segala et al., 1998 [23]	Sept. – Nov. 1992	Daily asthma attacks and asthma-like symptoms	Daily av. conc.: SO_2, suspended black particulates BS, suspended particulates with an aerodynamic diameter close to 10 μm (PM_{13}), NO_2	0, 1, 2, 3, 4, 5, 6	T, RH (daily av.)	Generalized estimating equations
Atkinson et al., 1999 [11]	Jan. 1992 – Dec. 1994	Daily emergency department visits for asthma and all other respiratory complaints	Daily av. conc.: NO_2, O_3, SO_2, CO, particles (as BS), PM_{10}	0, 1, 2, 3	T (daily max., min.); RH (06:00 h, 15:00 h)	Poisson regression analysis
Norris et al., 1999 [24]	Sept. 1995 – Dec. 1996	Daily emergency department visits for asthma	PM_{10} (daily av.), CO (daily av.); SO_2 (1-hr av.); NO_2 (daily max. 1 hr and daily av. conc.); O_3 (daily max. 8-hr)	0, 1, 2, 3, 4	T (daily av.), dew point T (daily av.)	Poisson regression analysis
Bobak and Leon, 1999 [25]	1986 – 1988	Low birth weight, stillbirth	Daily av. conc.: TSP, SO_2, NO_x (daily av.)	/	/	Logistic regression analysis
Gouveia and Fletcher, 2000 [26]	1991 – 1993	Mortality due to all causes, respiratory and pneumonia	PM_{10}, SO_2 (daily av. conc.); CO (max. 8- hr moving av.); O_3, NO_2 (max. hourly mean during the daily period)	0, 1, 2	T, RH, atmospheric pressure, rainfall, wind speed, wind direction (daily – av., max., min. level)	Poisson regression analysis
Gouveia and Fletcher, 2000 [12]	Nov. 1992 – Sept. 1994	Daily hospital admissions due to respiratory diseases	Daily av. conc.: PM_{10}, SO_2, NO_2, O_3, CO	0, 1, 2	T, RH (daily level – av., max., min.)	Poisson regression analysis
Fusco et al., 2001 [13]	Jan. 1995 – Oct. 1997	Daily hospital admissions due to respiratory conditions, acute respiratory infections, asthma	Daily av. conc.: particles, SO_2, NO_2, CO (daily integrated measure); O_3 (mean 08:00 h – 16:00 h)	0, 1, 2, 3, 4	T, RH (daily av.)	Poisson regression analysis
Thompson and Shields, 2001 [27]	Jan. 1993 – Dec. 1995	Treatment of acute asthma exacerbations in the emergency department	Daily av. conc.: PM_{10}, SO_2, NO_x, NO, NO_2, O_3, CO, benzene	0, av.0-1, 0-2, 0-3	T, rainfall (daily av.)	Poisson regression analysis
Hajat et al., 2002 [28]	Jan. 1992 – Dec. 1994	Number of consultations made at family practices due to upper respiratory disease	Daily av. conc.: SO_2, BS, NO_2, CO, O_3, PM_{10}	0, 1, 2, 3	T, RH (daily – max., min.)	Generalized additive model
Galan et al., 2003 [29]	1995 – 1998	Daily emergency department admissions due to asthma	PM_{10}, SO_2, NO_2 (daily av.); O_3 (av. of max. 8-hr)	0, 1, 2, 3, 4	T, RH (daily av.)	Poisson regression analysis
Zhang et al., 2006 [30]	Jan. 2001 – Dec. 2004	Daily mortality (all causes excluding accidents and injuries), cardiovascular and respiratory diseases	Daily av. conc.: O_3, NO_2, SO_2, PM_{10}	0, 1, 2, 3, 4, av. 0-1, 0-4	T, RH (daily – min., max., av.)	Generalized additive model
Conceição et al., 2009 [31]	Jan. 1994 – Dec. 1997	Daily records of mortality due to respiratory diseases	SO_2, PM_{10} (daily av. conc.); CO (greatest 8-hr moving av.); O_3 (24-hr peak)	1, 2, 3, av. 1-5	T (min.), RH (daily av.)	Poisson regression analysis
Nastos et al., 2010 [32]	2001 – 2004	Daily counts of childhood asthma hospital admissions	Daily av. conc.: PM_{10}	0, 1, 4	/	Poisson regression analysis and logistic regression analysis
Mansourian et al., 2010 [33]	Mar. 2005 – Mar. 2006	Hospital admission due to respiratory diseases	Daily av. conc.: SO_2, NO_2, PM_{10}; CO (8-hr av.)	/	/	Poisson regression analysis
Hua et al., 2014 [14]	Jan. 2007 – Jul. 2012	Daily asthma hospital admission	Daily av. conc.: $PM_{2.5}$, BC, NO_2, SO_2	1, 3, 5	T, RH (daily av.)	Distributed lag model

Legend:
hr – hour; av. – average; conc. – concentration; max – maximum; min – minimum; NO_2 – nitrogen dioxide [$\mu g/m^3$]; SO_2 – sulphur dioxide [$\mu g/m^3$]; CO – carbon monoxide [$\mu g/m^3$]; PM_{10} – particulate matter of 10 micrometres in diameter [$\mu g/m^3$]; O_3 – ozone [$\mu g/m^3$]; BS – black smoke [$\mu g/m^3$]; BC – black carbon [$\mu g/m^3$]; TSP – total suspended particulates [$\mu g/m^3$]; T – temperature [°C]; RH – relative humidity [%].

Table 2: Overview of ecological spatial studies at the population level included in systematical analysis

Research	Observed health outcome	Exposure to outdoor air pollution – pollutants and spatial units of observation	Methods
Pikhard et al., 2001 [34]	Prevalence of respiratory outcomes (wheezing, asthma, dry cough)	SO_2 concentration measurement by passive samplers (2 week campaigns in October 1993 and February 1994). Kriging interpolation technique had been used/used for spatial distribution of SO_2 conc. (Results: map of log-term estimation of winter levels of SO_2 for each point = 10×10 m). The arithmetic mean of SO_2 concentration at the location of home and school.	Logistic regression analysis
Hwang and Chan, 2002 [35]	Daily clinic visits for lower respiratory tract illness	Daily av.: NO_2, SO_2, PM_{10}; hr max.: O_3; max. 8-hr running av.: CO. Spatial units of observation: 50 individual small communities in Taiwan (50 townships and city districts in Taiwan where ambient air monitoring stations of the Taiwan Air Quality Monitoring Network are situated).	Poisson regression analysis (1st step), Bayesian hierarchical modelling (2nd step)
Wolf, 2002 [36]	Hospitalization chronic rhinosinusitis	SO_2, NO_x, dust fall (TSP). Spatial units of observation: 85 administrative districts in Cologne, Germany all measurements are aggregated to these units. Preparing map for spatial variation of health and air pollution data on the level of administrative units by using GIS.	Ordinary least-squares (OLS) regression
Scoggins et al., 2004 [37]	Mortality circulatory and respiratory causes	Annual av. NO_2. Spatial units of observation: 1296 grid cells (3×3 km), Auckland region, New Zealand. Spatial units of observation: Annual av. NO_2 modelling conc. was converted from point-based grid coverage into 3 km by 3 km polygon grid coverage in ARC INFO and ArcView GIS. Polygon grid coverage conc. were converted to census area unit (CAU) conc. by calculating an area weighted av. conc. for all individual CAUs that overlapped more than one grid cell.	Separate logistic regression Models and Poisson regression analysis
Oyana and Rivers, 2005 [38]	Hospitalization and outpatient visits for asthma and gastroenteritis	$PM_{2.5}$ and other pollutants in vehicle emissions (e.g. NO_2) Spatial units of observation: Buffalo's west side, east side, parts of the downtown areas, and 4 geographic regions) which is second-largest city in New York State. Data processing, GIS mapping, and analysis were conducted in ArcGIS and Microsoft Excel. ClusterSeer 2.03 was used to implement cluster analysis and Diggle's model. Final map production was completed using Corel Draw 11.	Multiple-comparison test
Wilson, 2006 [39]	Hospital admissions due to respiratory diseases	PM_{10} Spatial units of observation: TAPM (The Air Pollution Model) annual concentration outputs are produced on a grid with 1,500 m resolution in Christchurch, New Zealand. This gridded annual data was input into a GIS and then interpolated using a regular spline with five points of interpolation and a 100 m grid resolution. The zonal statistics tool in spatial analyst was then used to calculate the mean concentration for each CAU (Census Area Unit).	Poisson distribution model
Albuquerque et al., 2007 [40]	Daily records on asthma in the primary health system	CO, SO_2, PM_{10}, O_3, NO_2. The daily records from the air quality monitoring program were provided by the State Environmental Secretariat (SEAMA) and State Environmental Institute (IEMA). The air quality program is managed by the IEMA, which is responsible for five automatic monitoring stations. Spatial units of observation: 27 primary health units in the municipality of Greater Vitória, Brasil. (The municipality was divided by district and the outpatient treatment rates were calculated using the DATASUS database 8 per age bracket and home address.) An asthma distribution map was developed for using ArcGis.	Pearson correlation coefficient

Research	Observed health outcome	Exposure to outdoor air pollution – pollutants and spatial units of observation	Methods
Wang, 2008 [41]	Cardio-respiratory mortality	O_3, NO_2, SO_2. The daily gaseous air pollutants conc. were aggregated to annual means. Data were obtained from 13 monitoring stations by the Queensland Environmental Protection Agency (QEPA), including 8 stations within Brisbane city and 5 around the city. Spatial units of observation: 162 statistical local areas (SLA) cover the whole of Brisbane, Australia. The average size of a SLA was approximately 8 km^2 (ranged from 0.4 to 184.8 m^2). GIS techniques were used in mapping the spatial patterns of annual average O_3, NO_2, SO_2 conc. at a SLA level.	Spatial distribution analysis, multivariable logistic regression model
Choi et al., 2009 [42]	Daily mortality due to natural and cardiovascular causes	Annual av. of total $PM_{2.5}$ mass. The data were provided by the U.S. Environmental Protection Agency. 1st source of data: Federal Reference Method (FRM) monitoring network; 2nd: Interagency Monitoring of Protected Visual Environments (IMPROVE) network; 3rd: three-dimensional regional scale air quality models such as the U.S. EPA Community Multiscale Air Quality (CMAQ) modelling system. Spatial units of observation: CMAQ (36 × 36 km), FRM (38 monitoring sites), IMPROVE 83 monitoring sites).	Map outdoor $PM_{2.5}$ air conc. (1st step), Poisson regression analysis (2nd step)
Orru et al., 2009 [43]	Mortality and hospitalization due to cardiovascular and respiratory diseases	PM_{10}, $PM_{2.5}$ (annual levels) (3 monitoring states) Spatial units of observation: Tallinn was divided into 84 sections according to neighbourhoods. Grid resolution: 200 × 200 meters. The annual levels of $PM2_{.5}$ were calculated for all 84 Tallinn sections using the average concentration of modelled grid cells in a section. The average concentration for each section was then assigned to all residents of that neighbourhood.	Exposure-response functions
Maheswaran et al., 2012 [44]	Incidence of ischemic and hemorrhagic stroke	NO_2 and PM_{10}. The modelled data had been produced for Greater London and were available at a 20×20 m grid point resolution. Spatial units of observation: 948 census output areas in south London. The distribution of pollutants across output areas in the study area is shown in maps (PM_{10} and NO_2).	Poisson regression analysis
Leem et al., 2015 [45]	Asthma attack, acute bronchitis	$PM_{2.5}$, PM_{10} (a one-month period of each season in 2010 and 2024; January, April, July, and October). CMAQ (Comprehensive Multiscale Air Quality) was used to simulate air quality over the Seoul Metropolitan Area for a one month period of each season in 2010 and 2024; January, April, July, and October. Spatial units of observation: 3538 cells (3×3 km), Seoul Metropolitan Area.	Epidemiology based exposure response functions
Wang et al., 2015 [46]	Lung function parameters: forced expiratory volume in 1 sec (FEV_1), forced vital capacity (FVC), peak expiratory flow (PEF)	NO_2, $PM_{2.5}$, PM_{10}, $PM_{2.5}$ soot (annual av.). Three 2-week measurements within 1 year were conducted at 40 (PM) and 80 (NO_2) locations in Netherlands. Spatial units of observation: spatial resolution of 1 × 1 km. LUR: estimate annual average air pollution concentrations at the participants' home addresses at birth and at the time of the lung function tests. They used linear regression analyses with natural log (ln)–transformed lung function parameters as the dependent variables to estimate associations between continuous lung function parameters and air pollution levels at the birth address and at the home address at the time of the lung function measurement.	Dispersion and land use regression (LUR) models (1st step), The Dutch dispersion model (2nd step), linear regression analyses with natural log (3th step)

Legend:
hr – hour; av. – average; conc. – concentration; max – maximum; min – minimum; NO_2 – nitrogen dioxide; SO_2 – sulphur dioxide; CO – carbon monoxide; PM_{10} – particulate matter of 10 micrometres in diameter; $PM_{2.5}$ - particulate matter of 2.5 micrometres in diameter; O_3 – ozone; GIS - geographical information system.

The meteorological parameters used in ecological spatial studies were temperature [35, 40, 42], relative humidity [40], wind speed and pressure [42].

Previous research of the outdoor air pollution's effect on children's health at the population level in Slovenia area

In Slovenia epidemiological ecological research has not yet been extensively implemented. First studies of this type are displayed in Table 3.

Table 3: Overview of epidemiological ecological studies of the outdoor air pollution's effect on children's health at the population level in Slovenia area

Research	Type of study	Observed health outcome	Exposure to outdoor air pollution		Meteorological parameters	Methods
			Pollutants and spatial units	Lag [day]		
Šimac, 2008 [47]	TSS	Daily admissions due to respiratory diseases on the primary level of health system	O_3 (1-hr av.)	/	T	non parametric t-test
Kukec, 2013 [48], Kukec et al, 2014 [49]	TSS	Daily number of first consultations on the primary level of health system for any respiratory disease	PM_{10}, SO_2, NO_2 (24-hr av. conc); O_3 (8-hr max. av. conc.)	0, 1, 2, 3, 4, 5	air T, RH	Poisson regression analysis
Kukec, 2013 [48]; Kukec et al., 2014 [50]; Kukec et al., 2014 [51]	SS	Daily number of first consultations on the primary level of health system for any respiratory disease	Winter and summer av.: PM_{10}, SO_2; annual av.: NO_2. Spatial units of observation: small units of observation for each observed pollutant. Small spatial units of study were defined using the evaluated level of outdoor air pollution (dispersion model for each point = 200X200m) and digital maps of local communities and settlements in the municipalities of the Zasavje Region.	/	wind direction and speed, air T, turbulence	Bayesian hierarchical models
Rems-Novak et al., 2014 [52]	TSS	Daily number of first consultations on the primary level of health system due to asthma	O_3 (daily max. av.) PM_{10}, NO_2 (daily av.);	0, 1, 2, 3, 4, 5	T, RH (daily av.)	Logistic regression model

Legend:
TSS – time-series study; SS – spatial study; hr – hour; av. – average; conc. – concentration; max – maximum; NO_2 – nitrogen dioxide; SO_2 – sulphur dioxide; PM_{10} – particulate matter of 10 micrometres in diameter; O_3 – ozone; T – temperature; RH – relative humidity.

Possible limitations in connection to defined associations between outdoor air pollution and its effects on children's health at the population level

In Table 4 possible limitations of scientific articles with ecological study design (both time-series and spatial studies) are presented.

Table 4: Possible limitations of analysed ecological studies researching the effect of outdoor air pollution on children's health

Limitation	Research
Time-series studies	
Usefulness and unavailability of environmental data as the input data for linkage with health data	Kukec et al., 2014 [49]
Usefulness of health data as the input data for linkage with environmental data	Nastos et al., 2010 [32]; Rems-Novak et al., 2014 [52], Kukec et al., 2014 [49]
Exposure misclassification	Nastos et al., 2010 [32]; Thompson et al., 2001 [27]; Zhang et al. 2006 [30]; Mansourian et al., 2010 [33]; Galan et al., 2003 [29]
To low station of outdoor air pollution monitoring and varying distances from monitoring sites	Atkinson et al., 1999 [11]; Bobak and Leon, 1999 [25]; Thompson et al., 2001 [27]; Rems-Novak et al., 2014 [52]; Hua et al., 2014 [14]
Lack of accuracy in the measurements of exposure	Galan et al., 2003 [29]
Short period of observation	Rems-Novak et al., 2014 [52]
Small sample of observed population	Rems-Novak et al., 2014 [52]
Observed population does is not representative for the whole population	Mansourian et al., 2010 [33]; Rems-Novak et al., 2014 [52]
Difficult to separate the independent effect for individual pollutant in outdoor air pollution and there health effect	Hua et al., 2014 [14]
Spatial studies	
Usefulness and unavailability of environmental data as the input data for linkage with health data	Kukec, 2013 [48]; Kukec et al., 2014 [50]; Beale et al., 2008 [53]; Stroh et al., 2007 [54]
Unavailability of input air pollution data (emission) for air quality modelling Questionable validity of available data	Oyana and Rivers, 2005 [38]; Kukec, 2013 [48]; Kukec et al., 2014 [50]
Adequatbility of air quality modelling technique for dispersion of outdoor air pollution	Kukec, 2013 [48]; Kukec et al., 2014 [50]
Exposure misclassification	Porta et al., 2008 [55]; Maheswaran et al., 2012 [44]; Kukec, 2013 [48]; Kukec et al., 2014 [50]
Missing data on any individual-level risk factors, missing data of exposure	Eitan et al., 2010 [56]; Wolf, 2002 [36]
Difficulty to separate the independent effects for individual pollutant in outdoor air pollution and their health effect	Oyana and Rivers, 2005 [38]; Kukec, 2013 [48]
No control over the effects of all confounders	Oyana and Rivers, 2005 [38]; Kukec, 2013 [48]; Leem et al., 2015 [45]

DISCUSSION

The progress of research in the field of outdoor air pollution's effect on children's health at the population level

The research of outdoor air pollution's effect on children's health started in 1977. At that time all research was made at individual level and only in 1996 [22] and later in 1998 [10, 23] the first articles that were made at population level were published. These three studies were of ecological time-series design, where the association between outdoor air pollution and asthma and other respiratory symptoms are investigated. Previous research was made on the whole population [57, 58]. First ecological spatial studies were published after 2000 [34-36], where the association between outdoor air pollution and respiratory diseases was studied as time-series studies for the first time.

Methods of defining the association between outdoor air pollution and children's health at the population level

The effect of outdoor air pollution on children's health has been frequently studied in epidemiological studies; at the individual level (cross-sectional studies, case control studies and follow-up studies) and at population level (ecological studies). Overview of our review article shows that observed health outcomes in epidemiological ecological studies were frequent hospital admissions and visits to prime care due to respiratory diseases [10-14, 24, 27-29, 32, 33, 35, 38-40, 43, 47-52] and rarely cardiovascular diseases [43]. Up to 1990 in the epidemiological researches only outdoor air pollution's effect on respiratory diseases was observed [5].

In ecological studies PM_{10}, SO_2, NO_2, O_3 and CO were commonly observed pollutants for estimation of exposure to outdoor air pollution. The first reason (for inclusion in monitoring) is their effect on health [9, 59] and secondly, they are the most common outdoor air pollutants [8]. For measurements of PM_{10}, NO_2 and SO_2 in outdoor air daily average concentrations were used. For measurements of O_3 and CO daily maximum 8-hour or 24-hour concentrations were used. The most frequently used lags for health effects in time-series studies were from zero up to three or five days from the exposure. Daily data collecting of meteorological factors (air temperature and relative humidity) have been frequently used in time-series studies. Rarely measured meteorological parameters were dew point temperature, atmospheric pressure, rainfall, wind speed and direction of wind.

Poisson regression analysis was frequently used statistical method of defining the association between outdoor air pollution and children's health effects in time-series and spatial studies. Generalized additive model, logistic regression model, distributed lag model and generalized estimating equations were also used in singles time-series studies. Logistic regression analysis, Bayesian hierarchical model, multivariable logistic regression model and epidemiology based exposure response functions were also used in singles spatial studies.

Spatial units of observation in spatial studies were administrative units [35, 36, 38, 40, 41, 44] or small area units [34, 37, 39, 45, 46, 48].

Previous researches of the outdoor air pollution's effect on children's health at the population level in Slovenia area

In Slovenia methods of time-series and spatial studies have not yet been very extensively used. In time-series studies different methods of defining the association between outdoor air pollution and health effects have been used such as non-parametric [47], logistic regression model [52] and Poisson regression analysis [48, 49]. In Slovenia three spatial studies were made on the case of Zasavje region [48, 50, 51], two of them used Bayesian hierarchical models [48, 50]. In previous study [60] in Koper municipality maps with spatial distributions of observed health outcomes were prepared. The most important reasons for the rare use of ecological studies in Slovenia is uselessness and unavailability of environmental and health data for linkage analysis [48, 49, 52].

Limitations and strengths of our review

In our study only free full text available studies were overviewed. Due to this limitation not all studies in this research field had been overviewed.

On the other hand, this study has its strengths. Firstly, our overview of methods of association between outdoor air pollution and health effects on children focused on the methods used at population level. Several previous reviews included researches both at individual and population level and their results, not methods. Secondly, the overview of all ecological studies in Slovenia with used methods was included. The last strength of our research, a list of limitations of time-series and spatial researches was prepared.

Future researches

For future research ecological studies with pollutants that have already known effects on the whole population should be investigated on children. One of the investigated pollutants should be ultrafine particles; the effect of this air pollutant has already been investigated with ecological time trend studies for the whole population but not for children [61-63]. In future, special care should be given to data-collecting so that the data will be useful for linkage environmental and health data on population level [64]. Special challenge presents the preparation of hazard analysis at individual level. For hazard analysis of outdoor air pollution exposure fixed monitoring sites have been used. However, for improve exposure assessment in the future spatial investigation with exposure assessment in the small spatial units is necessary. Hazard analysis on pollutant's health effects should be made because of simultaneous exposure to mixture of different pollutants. An individual is exposed to different harmful factors from the environment with different effects. Because of lag time, slow and lasting effect they are usually hard to determine, because of their dispersion in the environment are also hard to control and are unpredictable. That is why in the future multiple effects [65], synergistic effect [66] and antagonistic effects [66] should be taken into account.

CONCLUSION

In conclusion, the most frequently used method of defining the association between outdoor air pollution and children's health effects in time-series and spatial studies was Poisson regression analysis. The respiratory diseases were frequently observed health outcomes, gaseous pollutants and particulate matter were frequently monitored pollutants in ecological studies. Exposure misclassification was the most frequent and repeated limitation in ecological studies.

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INSTRUCTIONS FOR AUTHORS

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Original research articles: Research articles of 6,000-10,000 words (10 – 18 manuscript pages) in length, with tables, illustrations and references, in which hypotheses are tested and results. Research articles report on significant and innovative achievements, approaches and should exhibit a high level of originality.

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Governmental initiatives: Reports on new or existing policies, governmental initiatives and programmes, have up to 2,000 words.

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Cover letter: Cover letter should contain:

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2. Full name(s) of the author(s).
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[1] Bhatt Siddharta M. Energy audit case studies II - Air conditioning (cooling) systems. Appl Th Eng. 2000; 20: 297-307.

[2] American college of physicians. Clinical Ecology. An Int Med 1989; 111:168-78.

[3] Vivian VL, ed. Child abuse and neglect: a medical community response. Proceedings of the first AMA national conference on child abuse and neglect. 1984 Mar 30-31; Chicago. Chicago: American Medical Assotiation, 1985.

[4] Mansfieid LW. How the nurse learns which imbalance is present. V: Moidel HC, Sorensen GE, Giblin EC, Kaufman MA, eds. Nursing care of the patient with medical-surgical disorders. New York: Mc Grow-Hill, 1971: 153-60.

[5] Evaluation of the European Agency for Safety and Health at Work: http://osha.europa.eu/publications/other/20010315/index_1.htm (20. 12. 2006).

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