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Issues in studies on the causal role of perinatal factors and the occurrence of asthma in children.

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Summary

In **chapter 1**, the rationale for this PhD project is discussed. Reviewing literature on asthma occurrence in childhood and early life exposures (e.g. perinatal exposure to antibiotics and exposure to environmental tobacco smoke) revealed that epidemiologic etiologic studies on the same exposure-outcome relationship yielded results with large differences in the estimates or even contradicting results. The explanations assigned to this were generally related to issues with the data such as a small sample size, selection bias, residual confounding, etc. Design issues were rarely mentioned, except when discussing the choice for either a case-control study or a cohort study.

Study design is interpreted in different ways depending on which epidemiological textbook is consulted. In some textbooks study design is interpreted as merely the designing of the collection of the data and the selection and sampling of the study population. In other textbooks, study design is more elaborated including also the designing of the study object and the designing of the data processing ('analysis'). Reporting guidelines and guidelines for conducting epidemiologic research do stress the importance of the reporting of all aspects of study design, but they do not go into the defining of these aspects.

Conducting scientific research starts with the identification of a relevant gap in scientific knowledge. A research question is then formulated, aiming for filling this gap in knowledge. Subsequently, a study is designed starting with the translation of this research question into an appropriate theoretical design, i.e. the object of research. The theoretical design is, together with the design of data collection and the design of data processing ('analysis') an essential element of study design. In epidemiological research, the theoretical design encompasses the formulation of an occurrence function (a mathematical function relating the outcome studied to the determinant(s)) and the specification of the domain (i.e. that part of the theoretical population to which the results pertain). In order to lead to valid results, it is crucial that the design of data collection as well as the design of data processing matches with the theoretical design. However, up until now, theoretical design has been undervalued in the training of epidemiological researchers, has not been explicitly mentioned in guidelines for reporting and conducting epidemiologic research and as a consequence has hardly been considered in the discussion on the aspects of the quality of a conducted study.

Therefore, the aim of this PhD-project was to gain insight into the reporting of theoretical design, the use of theoretical design by researchers and the knowledgeability of authors with this concept. The presented concepts in this PhD-

project are applied in two epidemiologic etiologic studies (incidence-density studies). The focus was on epidemiologic etiologic research on the occurrence of asthma and early life exposures and the aim was to add to the discussion on the relevance of theoretical design in epidemiologic etiologic research in general. In order to be able to interpret the findings in this PhD-project, the framework (study design for epidemiologic etiologic research) used in the thesis is presented.

The definition of study design often only refers to the design of data collection without referring to theoretical design. Additionally a hierarchy is assigned to the design of data collection (type of study) indicating the preferred 'design', for example the preference to conduct a cohort study when conducting a randomized controlled trial (RCT) is unfeasible or unethical. However, this approach does not have a univocal link with the research question of the study nor with the underlying theoretical design. In the introduction, the aspects of study design for epidemiologic etiologic research are presented with a univocal link to the research question.

In **chapter 2**, a critical appraisal is presented on the reporting of theoretical design in articles published in scientific peer reviewed journals indexed in the PubMed database on the association between early life antibiotic use and the occurrence of asthma. The reporting of theoretical design was compared between articles published before and after the publication of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement. In total, 63 articles were included for critical appraisal. In none of the articles a research question nor theoretical design was reported. The presence of seven key elements of theoretical design was therefore assessed (i.e. measure of occurrence, case (event or state) definition, conceptualization and operationalization of the exposure, temporal relation between outcome and exposure, confounders and effect modifiers taken into account and the domain of the study). Only 13 out of the 63 articles reported all key elements assessed. For 50 out of the 63 articles the temporal relation between outcome and exposure could be deduced from the information reported. For these 50 articles, a theoretical design was formulated (by HB and JW). For 15 articles, there was consensus about the underlying theoretical design being 'current occurrence as a function of past exposure'. For 24 articles there was consensus about the underlying theoretical design being 'future occurrence as a function of current exposure'. For the remaining 11 articles there was initially no consensus on what the theoretical design could be, because the extracted information reported in the article was ambiguous or even not reported at all. Comparing the articles published before and after the publication of STROBE revealed no marked differences in reporting, except for reporting of the measure of occurrence and the specification of effect modifiers taken into account.

In **chapter 3**, the results of an informal survey are presented on the role of theoretical design in the entire research process (before peer-review) leading to the publication of the article. A selection of thirty authors of the reviewed articles (described in chapter 2) was invited to fill in a questionnaire, of which 15 responded. One questionnaire was omitted because the author requested to not quote the answers. How authors perceived themselves to be knowledgeable with the concepts of theoretical design and causal theory was more diverse compared to how they perceived themselves to be knowledgeable with the concepts of confounding and effect modification (the vast majority perceived themselves to be moderately to extremely knowledgeable with the latter concepts). Focusing on theoretical design, only one author was able to formulate an occurrence function. However, once the authors were provided with occurrence functions to choose from, the vast majority selected 'current occurrence as a function of past or current exposure'. Half of these authors however, conducted their study as if the occurrence function is 'future occurrence as a function of current exposure' (based on the information extracted from the article). In all studies an epidemiologist was involved and almost all authors agreed to the changes made to the manuscript as a consequence of the reviewers' comments. According to the authors, none of the reviewers' comments were related to the reporting of theoretical design.

In **chapter 4**, a study is presented investigating the relationship between current (first) occurrence of asthma in children and antecedents of systemic antibiotic use in the first year of life with careful consideration of the temporal aspects of the determinant-outcome relationship. In this epidemiologic etiologic study, the presented concepts were applied. The design of data collection and data processing was matched with the theoretical design by conducting an incidence-density study. The incidence-density for asthma occurrence in children exposed to four or more courses of systemic antibiotics during the first year of life was almost twice the incidence-density for asthma occurrence in children exposed to less than four courses (although not statistically significant at the predominantly used α-level of 0.05). This association was much stronger (and statistically significant at the α-level of 0.05) in children reporting lower respiratory tract infections in the first year of life.

In **chapter 5**, a study is presented investigating the relationship between current asthma occurrence in children and antecedents of recent environmental tobacco smoke (ETS) exposure by conducting an incidence-density study. Additionally, the role of perinatal exposure to ETS and parental inhalation atopy as potential effect modifiers was assessed. Also here, the presented concepts were applied. An association was observed between the occurrence of a first doctor's diagnosis of asthma and recent exposure to ETS (1 year prior to diagnosis). There was no indication for effect

modification by perinatal exposure to ETS. The findings implied that exposure in early childhood, also outside the perinatal period, is associated with the occurrence of asthma.

In **chapter 6,** the findings are summarized and brought into a general perspective. We have the impression that theoretical design is underreported and not well known among researchers. The theoretical design is however the backbone for the design of data collection, the design of data processing and the interpretation of the study results. Explicit reporting of theoretical design is as a consequence of added value for increasing transparency and interpretability of a study. This was also reflected in the reviewers comments received for the manuscripts in this thesis. The reviewers of the manuscripts in chapter 2 and 3 commented that the work discusses an important aspect of reporting of epidemiological research. The reviewers of the manuscripts in chapter 4 and 5 perceived the methods section to be clearly written. However, the used terminology (i.e. population moments) was according to the reviewers probably difficult to understand for the readers of the journals. Therefore, epidemiologists should critically reflect on the approach (i.e. how to design, conduct and report) to their research. This approach is inextricably linked to the training of researchers. More specifically, epidemiologists should critically discuss the importance of the use and explicit reporting of theoretical design in research practice and how and when to introduce theoretical design in the training of researchers.

Samenvatting

In **hoofdstuk 1** wordt de rationale voor dit doctoraatsproject besproken. Bij bestudering van de literatuur over het optreden van astma bij kinderen en blootstellingen in het vroege leven (bv. perinatale blootstelling aan antibiotica en blootstelling aan omgevingstabaksrook) bleek dat epidemiologische etiologische studies over dezelfde blootstelling-uitkomstrelatie resultaten opleverden met grote verschillen in de schattingen van de effectmaten of zelfs tegenstrijdige resultaten. De verklaringen hiervoor hielden meestal verband met problemen met de gegevens, zoals een kleine steekproefomvang, selectiebias, residuele vermenging van effecten (confounding), enz. Kwesties betreffende de opzet werden zelden vermeld, behalve bij de bespreking van de keuze voor een case-control studie of een cohort studie.

Studieontwerp wordt op verschillende manieren geïnterpreteerd, afhankelijk van welk epidemiologisch leerboek wordt geraadpleegd. In sommige leerboeken wordt studieontwerp geïnterpreteerd als alleen het ontwerpen van de gegevensverzameling en de selectie en steekproeftrekking van de onderzoekspopulatie. In andere leerboeken wordt dieper ingegaan op studieontwerp en omvat het ook het ontwerpen van het studieobject en het ontwerpen van de gegevensverwerking ("analyse"). Richtlijnen voor rapportage en richtlijnen voor het uitvoeren van epidemiologisch onderzoek benadrukken wel het belang van de rapportage van alle aspecten van het studieontwerp, maar gaan niet in op de definiëring van deze aspecten.

Het verrichten van wetenschappelijk onderzoek begint met het vaststellen van een relevante leemte in de wetenschappelijke kennis. Vervolgens wordt een onderzoeksvraag geformuleerd, gericht op het opvullen van deze leemte in de kennis. Daarna wordt een studie ontworpen die begint met de vertaling van deze onderzoeksvraag in een passend theoretisch ontwerp, namelijk het object van onderzoek. Het theoretisch ontwerp is samen met het ontwerp van de gegevensverzameling en het ontwerp van de gegevensverwerking ("analyse") een essentieel element van het studieontwerp. In epidemiologisch onderzoek omvat het theoretisch ontwerp de formulering van een voorkomensfunctie (een wiskundige functie die de bestudeerde uitkomst in verband brengt met de determinant(en)) en de specificatie van het domein (d.w.z. dat deel van de theoretische populatie waarop de resultaten betrekking hebben). Om tot geldige resultaten te komen, is het van cruciaal belang dat zowel het ontwerp van de gegevensverzameling als dat van de gegevensverwerking overeenstemt met het theoretische ontwerp. Tot nu toe is het theoretisch ontwerp echter ondergewaardeerd in de opleiding van epidemiologische

onderzoekers, wordt het niet expliciet genoemd in richtlijnen voor het rapporteren en uitvoeren van epidemiologisch onderzoek en komt het als gevolg daarvan nauwelijks aan bod in de discussie over de aspecten van de kwaliteit van een uitgevoerd onderzoek.

Het doel van dit doctoraatsproject was daarom om inzicht te krijgen in de rapportage van theoretisch ontwerp, het gebruik van theoretisch ontwerp door onderzoekers en de kennis van onderzoekers over dit concept. De gepresenteerde concepten in dit doctoraatsproject worden toegepast in twee epidemiologische etiologische studies (incidentie-dichtheidsstudies). De focus lag op epidemiologisch etiologisch onderzoek naar het vóórkomen van astma en blootstellingen in het vroege leven en het doel was een bijdrage te leveren aan de discussie over de relevantie van theoretisch ontwerp in epidemiologisch etiologisch onderzoek in het algemeen. Om de bevindingen van dit proefschrift te kunnen interpreteren, wordt in dit hoofdstuk ook het in dit proefschrift gebruikte kader (studieontwerp voor epidemiologisch etiologisch onderzoek) gepresenteerd.

Studieontwerp wordt vaak gedefinieerd als het type onderzoek (ontwerp van gegevensverzameling) zonder referentie naar het theoretisch ontwerp. Daarnaast wordt een hiërarchie toegekend aan het ontwerp van de gegevensverzameling (type studie), waarbij het 'ontwerp' van voorkeur wordt aangegeven, bijvoorbeeld de voorkeur om een cohortstudie uit te voeren wanneer het uitvoeren van een gerandomiseerde gecontroleerde studie (RCT) onhaalbaar of onethisch is. Deze aanpak heeft echter geen eenduidige link met de onderzoeksvraag, noch met het onderliggende theoretisch ontwerp. In de introductie, worden de aspecten van studieontwerp voor epidemiologisch etiologisch onderzoek gepresenteerd met een eenduidige link met de onderzoeksvraag.

In **hoofdstuk 2** wordt een kritische beoordeling gepresenteerd van de rapportage van het theoretisch ontwerp in artikelen gepubliceerd in wetenschappelijke gepeerreviewde tijdschriften, geïndexeerd in de PubMed database, over de associatie tussen antibioticagebruik op jonge leeftijd en het optreden van astma. De rapportage van het theoretisch ontwerp werd vergeleken tussen artikelen gepubliceerd voor en na de publicatie van de Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) verklaring. In totaal werden 63 artikelen geïncludeerd voor kritische beoordeling. In geen van de artikelen werd een onderzoeksvraag of theoretisch ontwerp gerapporteerd. Daarom werd de aanwezigheid van zeven sleutelelementen van het theoretisch ontwerp beoordeeld (i.e. maat van voorkomen, definitie van het event (gebeurtenis of toestand), conceptualisering en

operationalisering van de blootstelling, temporele relatie tussen uitkomst en blootstelling, in aanmerking genomen confounders en effectmodificatoren en het domein van de studie). Slechts 13 van de 63 artikelen rapporteerden alle beoordeelde sleutelelementen. Voor 50 van de 63 artikelen kon de temporele relatie tussen uitkomst en blootstelling worden afgeleid uit de gerapporteerde informatie. Voor deze 50 artikelen werd een theoretisch ontwerp geformuleerd (door HB en JW). Voor 15 artikelen was er consensus over het onderliggende theoretische ontwerp 'huidig voorkomen als functie van blootstelling in het verleden'. Voor 24 artikelen was er consensus over het onderliggende theoretische ontwerp 'toekomstig voorkomen als functie van huidige blootstelling'. Voor de overige 11 artikelen was er aanvankelijk geen consensus over wat het theoretische ontwerp kon zijn, omdat de verkregen informatie uit het artikel dubbelzinnig was of zelfs helemaal niet werd gerapporteerd. Vergelijking van de artikelen die voor en na de publicatie van STROBE zijn gepubliceerd, liet geen duidelijke verschillen in rapportage zien, behalve wat betreft de rapportage van de maat van voorkomen en de specificatie van effectmodificatoren waarmee rekening is gehouden.

In **hoofdstuk 3** worden de resultaten gepresenteerd van een informele enquête over de rol van het theoretisch ontwerp in het gehele onderzoeksproces (vóór peer-review) dat leidt tot de publicatie van het artikel. Een selectie van dertig auteurs van de beoordeelde artikelen (beschreven in hoofdstuk 2) werd uitgenodigd een vragenlijst in te vullen, waarvan er vijftien hebben gereageerd. Eén vragenlijst werd weggelaten omdat de auteur verzocht de antwoorden niet te citeren. De kennis die auteurs dachten te hebben van de begrippen theoretisch ontwerp en causale theorie liep meer uiteen dan de kennis die zij dachten te hebben van de begrippen confounding en effectmodificatie (de overgrote meerderheid dacht matig tot zeer veel kennis te hebben van deze laatste begrippen). Wat het theoretisch ontwerp betreft, was slechts één auteur in staat een voorkomensfunctie te formuleren. Toen de auteurs echter de keuze kregen uit een aantal voorkomensfuncties, koos de overgrote meerderheid voor "huidig voorkomen als functie van vroegere of huidige blootstelling". De helft van deze auteurs voerde hun studie echter uit alsof de voorkomensfunctie "toekomstig voorkomen als functie van de huidige blootstelling" is (op basis van de informatie uit het artikel). Bij alle studies was een epidemioloog betrokken en bijna alle auteurs stemden in met wijzigingen in het manuscript als gevolg van de opmerkingen van de reviewers. Volgens de auteurs, hielden de opmerkingen van de reviewers geen verband met de rapportage van het theoretische ontwerp.

In **hoofdstuk 4** wordt een studie gepresenteerd waarin het verband wordt onderzocht tussen het huidige (eerste) optreden van astma bij kinderen en antecedenten van

systemisch antibioticagebruik in het eerste levensjaar, met zorgvuldige aandacht voor de temporele aspecten van de determinant-uitkomstrelatie. In deze epidemiologische etiologische studie werden de gepresenteerde concepten toegepast. Het ontwerp van de gegevensverzameling en -verwerking werd afgestemd op het theoretisch ontwerp door een incidentie-dichtheidsstudie uit te voeren. De incidentie-dichtheid voor het optreden van astma bij kinderen die gedurende het eerste levensjaar aan vier of meer kuren systemische antibiotica waren blootgesteld, was bijna tweemaal zo hoog als de incidentie-dichtheid voor het optreden van astma bij kinderen die aan minder dan vier kuren waren blootgesteld (hoewel niet statistisch significant op het gebruikelijke significantieniveau van 0.05). Dit verband was veel sterker bij kinderen die in het eerste levensjaar lagere luchtweginfecties rapporteerden (en statistisch significant op het significantieniveau van 0.05).

In **hoofdstuk 5** wordt een studie gepresenteerd waarin het verband wordt onderzocht tussen het optreden van astma bij kinderen en antecedenten van recente blootstelling aan omgevingstabaksrook (ETS) door middel van een incidentie-dichtheidsstudie. Daarnaast werd de rol van perinatale blootstelling aan omgevingstabaksrook en ouderlijke inhalatie-atopie als potentiële effectmodificatoren beoordeeld. Ook hier werden de gepresenteerde concepten toegepast. Er werd een verband waargenomen tussen het optreden van een eerste doktersdiagnose van astma en recente blootstelling aan omgevingstabaksrook (1 jaar voorafgaand aan de diagnose). Er waren geen aanwijzingen voor effectmodificatie door perinatale blootstelling aan omgevingstabaksrook. De bevindingen impliceren dat blootstelling in de vroege kinderjaren, ook buiten de perinatale periode, verband houdt met het optreden van astma.

In **hoofdstuk 6** worden de bevindingen samengevat en in een algemeen perspectief geplaatst. We hebben de indruk dat het theoretisch ontwerp onderbelicht en onbekend is onder onderzoekers. Het theoretisch ontwerp vormt echter de ruggengraat voor het ontwerp van de gegevensverzameling, het ontwerp van de gegevensverwerking en de interpretatie van de onderzoeksresultaten. Expliciete rapportage van de theoretische opzet is dan ook van toegevoegde waarde voor het vergroten van de transparantie en de interpreteerbaarheid van een studie. Dit kwam ook tot uiting in de commentaren van de beoordelaars van de manuscripten in deze thesis. Voor de manuscripten in hoofdstuk 2 en 3 werd opgemerkt dat een belangrijk aspect van de rapportage in het epidemiologisch onderzoek wordt besproken. De manuscripten in hoofdstuk 4 en 5 kregen positieve commentaren met betrekking tot de beschrijving van de gebruikte methoden. Echter, volgens de beoordelaars was de gebruikte terminologie (i.e. populatiemomenten) waarschijnlijk moeilijk te begrijpen

voor de lezers van de tijdschriften. Daarom moeten epidemiologen kritisch nadenken over de gehele aanpak (i.e. het ontwerpen, uitvoeren én rapporteren) van hun onderzoek. Deze aanpak is onlosmakelijk verbonden met de opleiding van onderzoekers. Meer in het bijzonder zouden epidemiologen moeten reflecteren over de wenselijkheid en het belang van het gebruik en de expliciete rapportage van theoretisch ontwerp in de onderzoekspraktijk en over de vraag hoe en wanneer theoretisch ontwerp in de opleiding van onderzoekers moet worden geïntroduceerd.

Chapter 1: Introduction

1.1 Rationale

The development, critical appraisal and application of epidemiological methods in health science research have always been a topic of interest at the department of Epidemiology and Social Medicine (currently the research group Social Epidemiology and Health Policy (SEHPO) within the department of Family and Population Health (FAMPOP) at the University of Antwerp). One of the areas of application has been research on respiratory health and in particular asthma. The research group was involved in international projects such as the European Community Respiratory Health Survey (ECRHS) and the International Study of Asthma and Allergies in Childhood (ISAAC). As a consequence of the growing interest in the role of early life exposures in the development of asthma, a research project was set up within the department: the Prospective project on the Influence of Perinatal factors on the Occurrence of Asthma and Allergies (PIPO). In this project a vast amount of data on early life exposures relevant for the development of asthma and allergies was gathered. Among these are early life antibiotic exposure as well trans-placental as via direct use,[1,2] exposure to tobacco smoke (ETS, also before and after birth), breastfeeding,[3] exposure to house dust mites,…. The potential role of early life exposure to antibiotics and to ETS seemed to be challenging as it is clear that these exposures might be already important for the development of immune related response during pregnancy as well as immediately after birth. Moreover, both are attractive targets for prevention. It is therefore important to document in detail exposures during the whole perinatal period (before and after birth). This would enable the (challenging) assessment of the appropriate temporal aspects, taking into account not only whether the exposure occurred in the first year of life and/or during pregnancy, but also the timing within the pregnancy and during the first year of life (before or after the start of the first symptoms).

Before starting our own research, literature on the relationship between early life exposures and the occurrence of asthma was reviewed. The results of studies published on the role of antibiotics were however often contradicting. Authors explained these differences as related to issues with the data (small sample size, selection bias, residual confounding,…). Design issues were only mentioned when discussing the choice between either a case-control study, a cross-sectional study or a cohort study, or (implicitly) when discussing so-called 'reversed causation'. Our interest in the methodological aspects of study design (including theoretical design, the

designing of how the data were collected, as well as the design of data processing) led to the question whether the approach to the study design in all its aspects could have led to issues of validity in the reviewed articles, in particular and more in general of the validity of medical scientific research. Therefore, it was decided that in this PhD-project the focus would be on epidemiologic etiologic research on the relationship between the occurrence of asthma and exposure to early life factors, with a special interest in the methodological aspects related to the approach to study design in all its aspects

Conducting a study starts from identifying a relevant gap in scientific knowledge and formulating an appropriate research question to fill in this gap. This research question should then lead to the (univocal) design of a study in which all important aspects are conceptualized and delineated. For epidemiological research questions, the researchers should be knowledgeable with all the important epidemiological concepts. However, for many basic (fundamental) concepts, different definitions can still be found in widely used textbooks. For example, the formula for incidence rate in 'A dictionary of epidemiology' (International Epidemiological Association, IEA) presented, is:[4]

$$Incidence\ rate = \frac{Number\ of\ new\ events\ in\ a\ specified\ period}{Average\ number\ of\ persons\ exposed\ to\ risk\ during\ this\ period} x10^3$$

In 'Epidemiology: An introduction' (World Health Organization, WHO) the formula for incidence rate presented is:[5]

$$Incidence\ rate = \frac{Number\ of\ subjects\ developing\ disease}{Total\ time\ experienced\ for\ the\ subjects\ followed}$$

Imprecise, ambiguous definitions of basic (fundamental) concepts can lead to different interpretations. The way a study is conducted and eventually is reported in an article depends on how these concepts are defined and interpreted by the researcher. As a consequence the quality of the conducted research depends on how the fundamental concepts are defined and interpreted.

Quality of research and research waste are general topics of concern that are not restricted to epidemiology or medical scientific research. Publication pressure (sometimes even leading to scientific misconduct) and sloppy science contribute to research waste. It is clear that applied research in contrast to fundamental research is more prone to this, as in applied research fundamental concepts are difficult to define. Another aspect leading to research waste, as pointed out by Ioannidis, is the lack of proper training of clinicians and scientists in methods and design.[7-8] Physicians for example can often practice medical research without formal training in quantitative research methods.

The growing concern with respect to the quality of both the conduct and the reporting (even in peer reviewed journals) of epidemiological research, called for the development of guidelines such as the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE, 2007) and more recently the Responsible Epidemiological Research Practice (RERP, 2018).[9,10] These guidelines discuss important elements of conducting epidemiological research such as study design, however without defining them. They do not go deeper into the technical aspects of study design, probably assuming that these aspects are well known by trained researchers.

For decades there has been discussion on the concept of study design, and this discussion has not ended yet.[6] This concept is up until now ill-defined and several interpretations can be found. Whereas in some textbooks study design is interpreted as merely the designing of the collection of the data, the selection of the study population, etc., in other textbooks there is much more elaboration on what the different key elements of study design are.

Study design encompasses the theoretical design, the design of data collection and the design of data processing.[11] Theoretical design is a concept that was introduced in epidemiology already in the 80's of last century by Miettinen, referring to it as 'object' design.[12] This object design encompasses the occurrence function (according to Miettinen epidemiologic research is occurrence research) of the study as well as the defining of the domain (referring to the part of the theoretical population to which the results can be applied on in a theoretical context). It is an essential aspect in the designing of a study, since it serves as the backbone for the design of data collection and the interpretation of the study results. In spite of the fact that according to STROBE all aspects of study design should be reported, theoretical design is rarely mentioned in the reporting of epidemiological studies and as a consequence up to now hardly considered in the discussion on the quality of research.

Therefore, the focus in this PhD-project is on theoretical design in epidemiologic etiologic research as applied in studies on the relationship between asthma occurrence and early life exposures. In this PhD-thesis we interpret epidemiologic etiologic research as research into the causes of illness, not into the causal consequences of an intervention (intervention research). In order to frame the work presented in this PhD-project, a status quaestionis of study design in epidemiologic etiologic research (definition, interpretation and prevailing dogma's) and the essential aspects of study design for epidemiologic etiologic research are first presented in what follows.

1.2 The concept of study design in epidemiologic etiologic research: status quaestionis

Study design is defined, in the most recent version (sixth edition) of 'A dictionary of epidemiology' published on behalf of the International Epidemiological Association (IEA), as:[4]

> *"The 'architecture', 'anatomy', or 'physiology' of a study: its structure and procedures, the specific methods, details on the selection and management of the study population (e.g., follow-up), time frame, and ethical decisions…."*

In the second edition (the first edition is untraceable and is no longer published) of 'A dictionary of epidemiology', study design was defined as:[13]

> *"The procedures and methods, predetermined by an investigator, to be adhered to in conducting a research project."*

Examination of a selection of epidemiological textbooks, reveals that study design is defined in various ways. In these textbooks, 'study design' is defined as the type of studies (the way of collecting information) that can be chosen when conducting epidemiologic research, the type of study population, the way the study population is sampled, the way exposures have been allocated, etc. Examples of commonly used 'so-called' study designs from these textbooks are: randomized controlled trial (RCT), cohort study, (nested) case-control study, cross-sectional study, ecological study and case-series.[14-17]

Widespread is the idea that a hierarchy exists in the sense that for causal research, the RCT should be considered as the gold standard. If conducting a RCT is not feasible or unethical, the next best option is a cohort study as it resembles a RCT and a third best option is a (nested) case-control study. The case-control study is considered as an efficient version of the cohort study.[18] Other lesser options are cross-sectional studies and ecological studies. This approach fails to generate a univocal link between these procedures and the research question (the researcher may seem to have a choice in deciding the procedures for data collection). This 'RCT-dogma' finds its origin in clinical intervention research. The 'RCT design' is then generally (dogmatic) applied in causally oriented research, be-it irrespective of a research question guided theoretical design which is an occurrence function, taking into account all relevant temporal aspects within a prespecified domain. This 'RCT-dogma' in medical scientific research has already been discussed.[19,20] It has already been pointed out that RCTs do not

necessarily provide certainty and guarantee that the answers will be closer to the truth compared to other types of studies.[5]

In other textbooks, there is more elaboration on a more comprehensive conceptualization of study design. In these textbooks it is stated that study design is more than the procedures of selecting the study population and collecting the data (cf. supra) and refers also to a theoretical design or design of the object (the fully specified occurrence function in a predefined domain) as a corner stone for the entire study design.[11,12]

Study design is then completed by designing the way information will be collected in representatives of the predefined domain, as well as by designing the way the collected information will be processed in order to closely match with the theoretical design and allowing to validly answer the underlying research question.

1.3 A framework for study design in epidemiologic etiologic research

In what follows the framework (including all important aspects of designing epidemiologic etiologic research) used in this thesis is presented and explained. We considered it necessary to dedicate a separate section to the framework we used for study design in epidemiologic etiologic research, as it is based on a compilation of elements presented by different authors, complemented with some own interpretations .

1.3.1 The research question

Designing a study starts with the formulation of a research question. In epidemiologic research, the research question encompasses three essential elements: (1) the outcome of interest (the occurrence of the studied illness), (2) the determinant(s) of interest (the (suspected) cause(s)) and (3) the domain of the research question (i.e. the part of the theoretical population for which the studied relationship is relevant within a theoretical context). What should be derivable from the research question, is the temporal relationship between the occurrence of the outcome and the presumed causal antecedents (determinant(s)). This temporal relationship is rarely explicitly elaborated on in textbooks, however it is important that it is already unambiguously specified in the research question.

Often, researchers formulate a hypothesis rather than a research question. A hypothesis however is by definition already an abstract concept, whereas a research question is to a certain level still concrete. Therefore, we prefer to start with the

formulation of a research question. However, regardless of formulating a hypothesis or a research question, the resulting abstract occurrence function (as part of theoretical design) should be the same.

1.3.2 Translating the research question into a theoretical design

The research question is translated into a theoretical design (by definition entirely abstract). The theoretical design is the combination of an occurrence function encompassing the object of a study (in causal research the strength of the association, or the derived attributable fractions) and a pre-specified domain. The domain is that part of the theoretical population to which the results can be applied on within a theoretical context. It should be pre-specified in order to select the appropriate (i.e. fitting the domain) study population. The core of the occurrence function is current occurrence of the outcome studied as a function of the determinant (the antecedent, the presumed cause) of interest (generally 'one at a time'). The measure of occurrence used should be a measure for 'current occurrence'. Occurrence of the outcome is therefore either a prevalence (for state-type illnesses) or an incidence density (for event-type illnesses). Cumulative incidence (the risk of developing the outcome after a period of follow-up) is a measure of future occurrence and should as a consequence not be used. The determinant of interest should be defined as antecedental to the outcome. Also included in the occurrence function are the (actual) confounders (in order to obtain an association conditional on the confounders) and the effect modifiers (including an interaction term in order to obtain modifier specific associations). Both confounders and modifiers are also assessed as antecedents of the outcome. The occurrence function is finally expressed in mathematical terms: characteristics are represented by variables/covariates, using the conventional symbols Y for the dependent variable, X_1 for the independent variable of central interest (the presumed cause) and $X_{2\text{-}i}$ for the independent variables representing the modifiers and confounders.

1.3.4 Design of data collection

Taking into account the theoretical design of the study (in all its details), the data are collected from a study base (sampling frame). In case of an event-type of illness the measure of occurrence should be current incidence density, defined as the number of events divided by the population time 'at risk' for the occurrence of the event for an observation period going to zero. Events and population time are (obviously) sampled from the study base in a non-zero observation period. Sampling the entire population time in a dynamic exposure experience and as a consequence measuring the absolute incidence density is in practice not feasible. Population time is therefore probed by sampling population moments within the same observation period as the period for

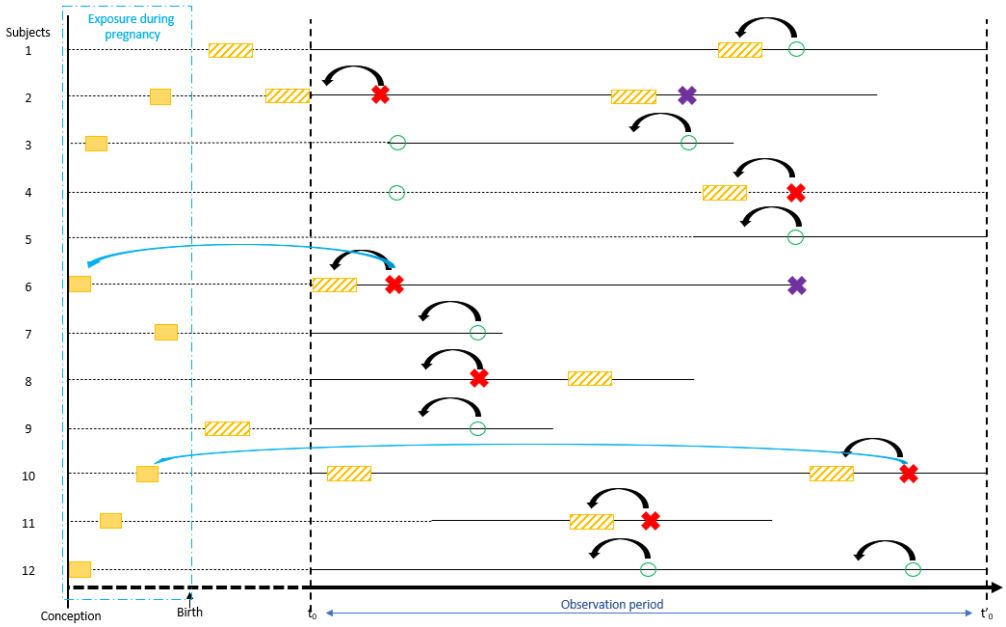
identifying the events. When this probing is independent from exposure, valid estimation of the incidence density ratio will however result. [21]
For example in figure 1, six events can be identified (when only first occurrence of the event is of interest). Of these, four events are exposed (taking the temporality into account, and the interest is in recent exposure). In total, eight population moments are sampled of which one is exposed. To be noticed, is that for example subject 2 is experiencing the event twice within the observation period, but if the interest is in incidence density of first events, the second event is not included. Subject 10 is exposed twice. For this subject only the most recent exposure is considered (if the interest is in recent exposure only). Also, a subject can be sampled twice as a population moment, as long as the subject is still at risk for developing the event (e.g. subject 12). In order to be able to assess exposure for population moments, the moment of sampling (timing within the observation period) must be taken into account.

When the interest is for example in exposure within a certain window, for example during pregnancy, the lag time (time between the occurrence of the first event and the moment of exposure) should be taken into account by introduction of an interaction term between lag time and exposure. For example, subject 6 and 10 in figure 1 were both exposed at the beginning of the observation period and both develop the event. If the interest is in exposure during pregnancy for example, then the lag time for subject 6 and 10 is different. This should be taken into account.

The same approach is used for assessing exposure to relevant characteristics that need to be taken into account as confounders and/or effect modifiers. We will however not elaborate on this.

In case of a state-type illness the prevalence odds is assessed as the number of individuals with the state-type illness at a pre-specified moment divided by the number of individuals without that state-type of illness at the same moment. In contrast to the incidence density assessment, the study base is not population time, but the population as such. As for the incidence density assessment however, a sample of the unaffected part of the study base (independent of exposure) allows for the valid assessment of the prevalence odds ratio. Information on the determinant(s) of interest, confounders and effect modifiers is then collected as antecedents (i.e. prior to onset).

Figure 1: *identification of events and sampling of population moments within an observation period and assessment of the exposure status as an antecedent.*



Exposure 1 assessed as a 'recent' antecedent before occurrence of the event or before sampling as a population moment.

Exposure 2 assessed as exposure during pregnancy. In this case the lag time (time between exposure and occurrence of the event or sampling as a population moment) can be taken into account, which is for example longer for subject 10 than for subject 6.

Event 1: event of interest occurring for the first time in the subject, regardless of the observation period.

Event 2: event of interest occurring (the same event as event 1) for the second time in the subject.

Population moment.

1.3.5 Design of data processing

Depending on the type of outcome, the data are processed in either a current incidence-density (for an event-type outcome) study or a current prevalence study (for a state-type outcome). In an incidence-density study the incidence-density ratio is estimated (conditional on the pre-specified confounders and also taking into account effect modifiers).[21] In a current prevalence study the prevalence odds ratio is estimated, also conditional on the pre-specified confounders and taking into account

effect modifiers. The appropriate statistical techniques are chosen for this. In case of a simple exposure-outcome relationship, a logistic regression is often appropriate. However, sometimes more sophisticated statistical approaches are required, but we consider this to be beyond the scope of this PhD-project.

1.4 Aim of the project

The general aim of this PhD-project is to add to the discussion on the relevance of theoretical design in epidemiologic etiologic research by gaining insight into the following aspects of the application of theoretical design: reporting in literature, use of theoretical design by researchers and knowledgeability of the concept among researchers.

The specific aims are:

1. To critically appraise the reporting of theoretical design and key elements of theoretical design in epidemiologic etiologic research on the relationship between the occurrence of asthma and early life antibiotic use.
2. To appraise the perceived knowledgeability of theoretical design among authors publishing research on the relationship between the occurrence of asthma and early life antibiotic use.
3. To apply the concepts (discussed and presented in the work for specific aim 1 and 2) in a study on the relationship between the occurrence of asthma and use of systemic antibiotics during the first year of life in children.
4. To apply the concepts (discussed and presented in the work for specific aim 1 and 2) in a study on the relationship between the occurrence of asthma and recent exposure to ETS (1 year prior to diagnosis) in children.

Since the aim of this PhD-project is to add to the discussion on the relevance of theoretical design in epidemiologic etiologic research, the empirical findings of specific aims 4 and 5 will not be discussed in detail in the conclusion. In the general discussion the reviewers' comments received regarding the use and reporting of theoretical design in the manuscripts will be taken into account.

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Chapter 2: Reporting of "Theoretical Design" in Explanatory Research: A Critical Appraisal of Research on Early Life Exposure to Antibiotics and the Occurrence of Asthma

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2.1 Abstract

"Theoretical design" comprises the development of an occurrence relation and the specification of the study domain. In explanatory research, the occurrence relation causally relates one determinant to the occurrence (of an event or a state) taking into account other relevant characteristics (confounders and modifiers). Conflicting results in explanatory research might be (partially) explained by differences in the "theoretical design" or by a mismatch between the "theoretical design" and the "design of data collection". In this critical review, the reporting of "theoretical design" is assessed in articles on the association between early life antibiotic use and the occurrence of asthma. Articles investigating a relationship between early life antibiotic use and the occurrence of asthma were searched in PubMed and systematically selected for critical review. The full text was read and important elements of study design were extracted (the research question/hypothesis, seven key elements of "theoretical design" (measure of occurrence, case (event or state) definition, conceptualization (and operationalization) of the exposure, temporal relation between outcome and exposure, confounders and effect modifiers taken into account and the domain of the study), the method of data collection and the method of data processing). A comparison was made between articles published before and after the publication of the "Strengthening the Reporting of Observational Studies in Epidemiology" (STROBE) statement (2007). Sixty-three articles were included for review. Thirteen articles reported the seven key elements of "theoretical design" that were questioned. No marked differences in reporting were observed pre- and post-STROBE. All articles reported some key elements of "theoretical design"; however, the reporting is not

structured and not linked to the concept of “theoretical design”. Conceptualizing, delineating and explicit reporting of “theoretical design” is quintessential for the quality and transparency of explanatory research.

2.2 Introduction

Conceptualizing, delineating and reporting all elements of study design (the design of the theoretical object, the design of the collection of the data and the design of the processing of the data collected) is quintessential both for the quality and for the interpretability of a study, especially when complex relationships between outcome(s) and exposure(s) are investigated. In epidemiological research, when specifying study design, researchers often merely refer to the “design of data collection” (e.g., cohort study, case-control study, cross-sectional study, …).[1–5] However, the design of how the data are collected should be considered as only a (be it an important) part of study design. Study design comprises three parts: the design of the “theoretical object” of the study (what will be studied and in what context), the design of how the data are collected (what kind of data will be collected and how will they be collected, i.e., defining the study population, the sampling procedures, and the measuring of the characteristics studied) and the design of how the data are processed (the main statistical methods used to process the data from the measurement of the characteristics to the operationalized variables used in the assessment of the association between outcome and exposure).

The development of the “theoretical design” is determined by an appropriate research question that should include the outcome under study, the exposure(s) of interest, and the domain of the study.[6] Central to the “theoretical design” is the translation of this research question/hypothesis into an occurrence relation. Focusing here only on explanatory research, the occurrence relation relates one determinant (the presumed cause) to the (frequency of) occurrence (of an event or a state) taking into account other relevant characteristics (extraneous to the causal pathway: confounders and non- extraneous to the causal pathway: effect modifiers). The application of a theoretical framework for causal inference, e.g., the theory of directed acyclic graphs (DAGs) or the sufficient-component cause model can be helpful as a tool for the selection of the relevant characteristics prior to the collection and/or exploration of the data.[7,8]

Already in the eighties of last century, Miettinen referred to the occurrence relation as being part of “object design”:[9]

"Designing the type of end result of an epidemiologic study means making the transition from an informal concept of the research problem to an express definition of the occurrence relation to be studied."

This involves designing the nature of the occurrence relation and the domain of the empirical occurrence relation. The nature of the occurrence relation includes: (1) the outcome state(s) or event(s); (2) the parameter of interest; (3) the determinant(s); (4) the time relation between outcome and determinant status; (5) modifiers and (6) potential confounders.[9]

The explicit formulation of this "object design" or "theoretical design", including the formulation of an occurrence relation and a domain, is crucial for the choice of an appropriate method of data collection and method of data processing.[6,9,10] In what follows both "object design" and "theoretical design" will be referred to as "theoretical design", since these are conceptually similar.

When it comes to the reporting of epidemiological studies, different guidelines have been published. One of these guidelines, the "Strengthening the Reporting of Observational Studies in Epidemiology" (STROBE) statement[11] refers to the importance of reporting the objectives of a study (note that these objectives are not objects as described by Miettinen) and advises to present key elements of study design early in the methods section or at the end of the introduction. In a publication by the Responsible Epidemiological Research Practice (RERP) working group of the Netherlands Epidemiological Society, a guideline also referring to the need for an appropriate design was formulated.[12] This guideline, however, does not discuss details on technical aspects of epidemiological research (e.g., study design).

Considering the importance of the formulation of a relevant research question/hypothesis (advised by both STROBE and RERP) and the translation hereof into an explicit "theoretical design" (based on both an occurrence relation and a domain) for the designing of data collection and the designing of data processing, these fundamental elements of study design should be explicitly reported in epidemiological literature.[6,9,12,13]

The explicit formulation of an appropriate "study object" or "theoretical design" (both in the setting up of a study as well as in the reporting) is essential for the justification of the choice for the method of data collection and method of data processing.[6] Diverging results in research on complex exposure-outcome relationships might be (at least partially) explained by differences in the object of the study (different underlying 'theoretical designs') or by a mismatch between the object of the study ("theoretical design") and the way data are collected ("design of data collection"). The relationship between early life antibiotic use and the occurrence of asthma is such a complex exposure-outcome relationship and studies on this relationship have been showing

diverging results.[14,15] Several studies refer to reversed causation and confounding-by-indication as a possible explanation of the associations found between early life antibiotic use and the occurrence of asthma.[16–18] Asthma is a complex disease whose etiological mechanisms are not fully understood. Potential risk factors for asthma can act in the prenatal period, at birth and later in life. As a consequence, the unraveling of the etiology of asthma is challenging, since historical reconstruction (i.e., prior to asthma onset) of relevant exposures, risk factors and other relevant characteristics is not straightforward. Early life exposures might also not be as relevant for adult onset asthma as for childhood onset asthma as the time interval between the exposure and the outcome differs.

So far, existing methodological reviews on the association between early life antibiotic use and the occurrence of asthma, mainly focus on the design of data collection (cohort, case control, retrospective, prospective, …) and on how outcome definition for asthma, reversed causation and confounding-by-indication could affect this association.[16,17,19] Marra et al. advise in their review that future research on the association between early life antibiotic use and the occurrence of asthma should address other methodological flaws.[16] To our knowledge, no methodological reviews have been conducted focusing on the explicit reporting of "theoretical design" in explanatory research. Therefore, the aim of this critical review was to gain insight (factual knowledge) in the "current" reporting of a "theoretical design" in (English language) articles published in scientific peer reviewed journals indexed in the PubMed database on the association between early life antibiotic use and the occurrence of asthma and in to what extent differences in reporting of "theoretical design" can be observed between articles published before and after the publication of STROBE (2007).

2.3 Methods

2.3.1 Theoretical design

The (factual) research questions and corresponding theoretical designs are:

1. To what extent do researchers explicitly report a "theoretical design" in (English language) articles published in scientific peer-reviewed journals indexed in the PubMed database on the association between early life antibiotic use and the occurrence of asthma?

   "Theoretical design": Current explicit reporting of "theoretical design" as an "intercept only" function in (English language) articles published in scientific

peer reviewed journals indexed in the PubMed database on the association between early life antibiotic use and the occurrence of asthma.

2. To what extent does the use and reporting of "theoretical design" differ between articles published before vs. after STROBE (2007)?

   "Theoretical design": Current explicit reporting of "theoretical design" as a function of the era of publication (pre- vs. post-STROBE) in (English language) articles published in scientific peer reviewed journals indexed in the PubMed database on the association between early life antibiotic use and the occurrence of asthma.

2.3.2 Search strategy and selection of articles

A search in PubMed was conducted using the following medical subject headings (MeSH): "asthma" AND "antibiotics". To assess current explicit reporting of "theoretical design", "current" was defined as the era including all (English language) articles published in a scientific peer reviewed journal indexed in the PubMed database on the association between antibiotic use and the occurrence of asthma. The search was performed on the 7th of January 2019 and a selection was made from all articles resulting from the search in PubMed reaching over the predefined period. This selection was based on a three-step procedure.

First, in the screening phase, the title and the aim or research question/hypothesis reported in the abstract was read. Articles were excluded if it was clear from the information provided that the interest was not in investigating a causal relationship between early life antibiotic use and the occurrence of asthma. If this information was ambiguous or missing in the abstract, the article was not excluded in this first step. Secondly, the full abstract of the remaining articles was read. In this step, articles were excluded if (1) the articles did not report on a study investigating a causal relationship between early life antibiotic use and the occurrence of asthma, (2) the causal relationship between early life antibiotic use and the occurrence of asthma was investigated in vitro or in animals and (3) no full (English language) text of the article was accessible or available. Articles remaining after this screening phase were assessed for eligibility by reading the full text of the articles. After reading the full text of the remaining articles, articles were excluded if they were not primary publications (e.g., review articles) or they did not report on a study investigating a causal relationship between early life antibiotic use and the occurrence of asthma after all. This implies that the abstract was missing or the information provided in the abstract was incomplete or ambiguous. The remaining (English language) articles reporting on a

study that investigated a causal relationship between early life antibiotic use and the occurrence of asthma were included for review.

No abstract domain was specified for this critical review. The aim was to gain factual knowledge about current explicit reporting of a "theoretical design" in (English language) articles published in scientific peer reviewed journals indexed in the PubMed database on the causal relationship between early life antibiotic use and the occurrence of asthma (observation period from June 1998 until the 7th of January 2019, i.e., the era between the publication of the oldest article indexed in PubMed on the association between early life antibiotic use and the occurrence of asthma and the day the search was performed). Occurrence of asthma was not restricted to occurrence in childhood, but articles investigating occurrence of asthma in adolescence or adulthood were included as well.

2.3.3 Extraction of relevant information

For every article selected, the following information (table 1) was extracted after reading the full text twice (HB).

2.3.4 Critical review of the articles

As study design starts with the formulation of an appropriate research question/hypothesis which is then translated into a "theoretical design", the presence of such an appropriate research question/hypothesis referring to the outcome, the exposure(s) and the domain of the study was first assessed.

Secondly, the presence of an explicitly formulated "theoretical design" (including the occurrence relation, an explicit temporal structure and the formulation of the domain of the study) was assessed. When no explicit "theoretical design" was reported, the presence/absence of seven key elements of "theoretical design" was discussed (HB and JW):

(a) Measure of occurrence.

(b) Case (event or state) definition (for asthma).

(c) Conceptualization and operationalization of the exposure.

(d) Temporal relation between outcome and exposure derivable from the research question/hypothesis or from the combination of the case (event or state) definition and conceptualization of the exposure. This means that by reading the research question/ hypothesis or the reported case (event or state)

definition and the conceptualization of the exposure, it should be clear how the exposure was situated in time in relation to the outcome (as an "antecedent" or as a "starting point").

(e) Confounders that were considered.

(f) Effect modifiers that were taken into account.

(g) Domain of the study.

2.3.5 Derivation of the theoretical design

For all articles where the temporal relation between the outcome and the exposure could be derived from the aim or from the case (event or state) definition and the conceptualization of the exposure, a "theoretical design" was formulated. In order to formulate this "theoretical design" all information extracted from the article (specified in table 1) was considered. The "theoretical design" was formulated first by HB and JW separately. In case of divergent interpretations, the "theoretical design" was discussed and assigned "in consensus".

Table 1: *Explanation on what information was extracted and how this information was extracted from the articles*

Information extracted	**Explanation**
1. Journal	Needs no further explanation.
2. Year of publication	Needs no further explanation.
3. Title of the article	Needs no further explanation.
4. Aim of the study	The aim of the study reported at the end of the introduction section was copied.
5. Research question or hypothesis of the study	The research question/hypothesis reported was copied.
6. Whether the authors mentioned the word(s) '(study) design' in the text (including the location in the text)	Needs no further explanation.
7. The case (event or state) definition for asthma	Needs no further explanation.

8. Scientific T_0 (and whether this was explicitly mentioned) and (theoretical) temporal aspects	T_0 refers to scientific (reference) time. If the interest is in studying 'future occurrence of asthma as a function of current exposure to antibiotics', then the moment of realization of exposure is the reference (T_0). If the interest is in studying the 'current occurrence of asthma as a function of a history of antibiotic use', then the moment of occurrence (asthma onset/diagnosis) is the reference (T_0). If the T_0 was not explicitly mentioned, a decision was made after discussion (HB and JW) on what T_0 could have been after taking into account the aim or research question/hypothesis of the study, the measure of occurrence, the method of data collection, the method of data processing and the abstract (theoretical) temporal aspects of confounders. The following example refers to the process of defining T_0: • **Aim:** *"…to assess what the association is between the exposure to antibiotics in the first year of life and later risk on asthma occurrence by age 7."* • **Measure of occurrence:** future incidence • **Method of data collection:** longitudinal study • **Method of data processing:** cox proportional hazards regression • **Abstract temporal aspect of confounders:** confounders were assessed in the first year of life of the child T_0 in this case would be at the age of 1 year. Incident cases of asthma would be detected from this point in time and onwards (= future occurrence).
9. The measure of disease occurrence (e.g.: prevalence, incidence) and the abstract (theoretical) temporal aspect hereof (current-, future-)[a]	Needs no further explanation.

10. The conceptualization of the exposure and the abstract (theoretical) temporal aspect hereof (past-, current-)[a]	The conceptualization of the exposure to antibiotics as reported by the authors was extracted. In case the exposure was conceptualized in multiple ways (e.g. antibiotic use during pregnancy, antibiotic use during the first year of life,…), all conceptualizations were extracted. Additionally the temporal aspect (past exposure to antibiotics if the interest was in the current occurrence of asthma or current exposure to antibiotics if the interest was in the future occurrence of asthma) was extracted from the article.
11. The operationalization of the exposure	Operationalization of the exposure to antibiotics refers to how the authors operationalized exposure to antibiotics when the data were processed. This could be for example dichotomous (exposed vs. non-exposed), but also in several categories of exposure (per class of antibiotic, per number of courses,…).
12. The measurement method for the exposure	The method used to assess the exposure to antibiotics in the study was extracted. This can be for example a questionnaire.
13. The measure of association	The estimate calculated by means of the statistical method applied to assess the strength of the association between antibiotic use and the occurrence of asthma was extracted from the article. This could be a hazard ratio, an odds ratio, an incidence density ratio, a causal fraction,….
14. Confounders taken into account (including the abstract (theoretical) temporal aspects)	All confounders taken into account and reported by the authors (including the timing of assessment) were extracted.
15. Effect modifiers taken into account (including the abstract (theoretical) temporal aspects)	All effect modifiers taken into account and reported by the authors (including the timing of assessment) were extracted.
16. The justification for the selection of confounders and/or effect modifiers	If any justification for the selection of confounders and/or effect modifiers (e.g.: selection based on a priori knowledge, selection based on the construction of a DAG[7], sufficient-component cause model[8],…) was reported in the article, this was extracted.
17. The domain of the study	The domain refers to the population to whom the results can be applied on.[6] After reading the full text, the domain of

	the study reported (either explicitly or implicitly) was extracted.
18. The design of data collection	The method used to collect the data for the study was extracted. This could be for example by means of a (birth) cohort, a case-control study,…. Example from a reviewed article: *"The Home Allergens and Asthma Study is a prospective birth cohort study of children with a parental history of asthma or allergies in the Boston metropolitan area."* In this study the association between antibiotic use and the occurrence of asthma was assessed by using data from a prospective birth cohort. Therefore the design of data collection is a prospective birth cohort.
19. The design of data processing	The main statistical methods used to process the data and to assess the association between antibiotic use and the occurrence of asthma was extracted from the article.
20. Whether there is any referral to a methodological paper or work supporting the used methods or referral to a reporting guideline	If any referral was made to a methodological article, a theoretical work or a reporting guideline supporting the applied epidemiological methods in the study or the reporting, this was indicated with 'yes'.

Notes: [a]Temporal aspects refer to the time structure between the occurrence of asthma and the exposure to antibiotics. In etiologic research, a causal relationship between an outcome and an exposure can only be assessed when the exposure occurred before onset of the outcome under study; Italic font indicates text quoted from an article.

2.3.6 Comparison based on the year of publication

The explicit reporting of a "theoretical design" (or the seven key elements (cfr. supra) of "theoretical design" in case no explicit "theoretical design" was reported) was compared between articles published before and after the publication of the STROBE statement in 2007 by categorizing the articles in two groups: articles published between 1998 and 2007 and articles published between 2008 and 2019.

2.3.7 Processing of the extracted information

For the presence of all reviewed elements (presence of the outcome, exposure and domain in the research question/ hypothesis, explicit reporting of "theoretical design", presence/absence of seven key elements of "theoretical design") absolute numbers are presented for all articles together. For the comparison based on the year of publication

(pre- vs. post-STROBE) absolute numbers and percentages were calculated per group. No statistical tests were performed as the aim was limited to the reporting of the facts in the selected articles (based on topic and era), and not to make any inference with respect to eventual differences found pre- vs. post-STROBE.

For each of the assessed elements of "theoretical design" (in case no explicit "theoretical design" was reported), two examples are provided in Appendix 3, one in case the assessed element was considered to be present and one in case the same element was considered absent. All examples are taken from the reviewed articles, but are anonymized. Also, for the derivation of "theoretical design" anonymized examples are presented in Appendix 4 to explain this process. Text quoted literally from the articles is written in italic and between quotation marks. Reporting was done in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[20]

2.3.8 Deviations from the methodology

Any deviations from the described methodology (cfr. supra) are indicated and explained in the results section.

2.4 Results

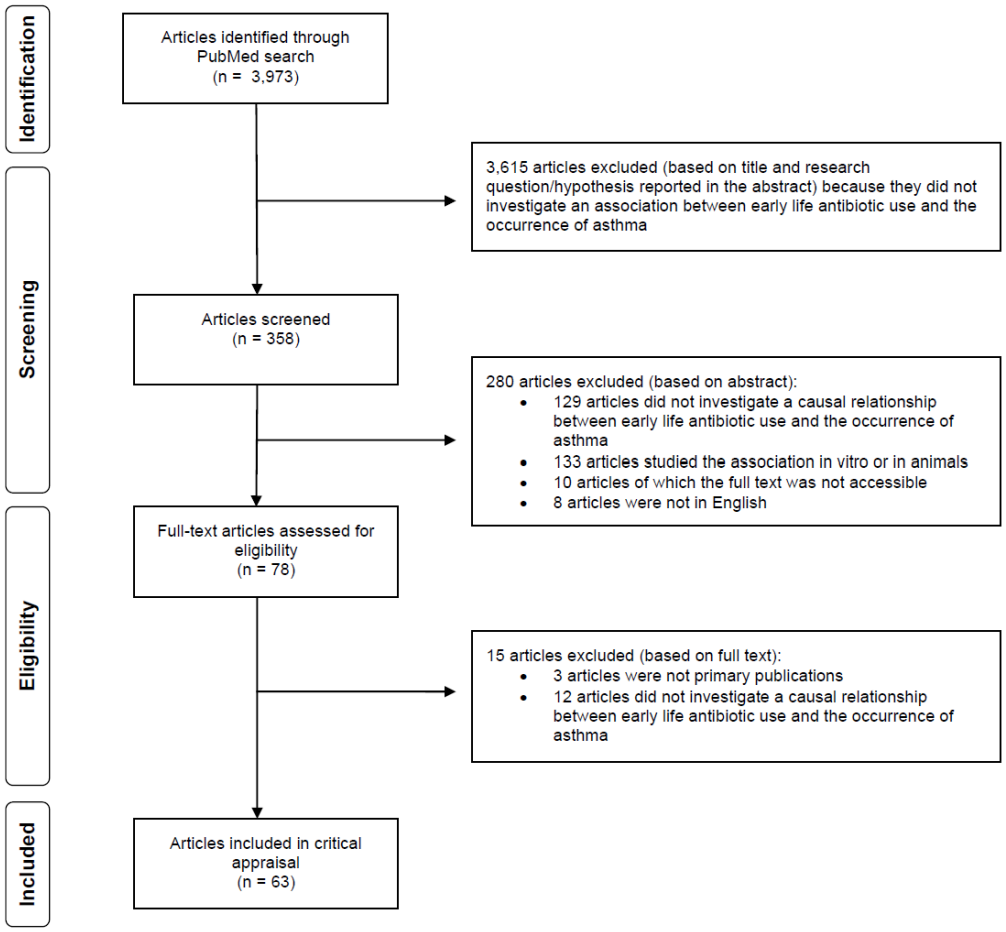
2.4.1 Selection of articles

The search in PubMed yielded 3973 articles. In figure 1 the selection procedure for the articles is presented. The observation period was defined as the era between the publication of the oldest article (investigating a causal relationship between early life antibiotic use and the occurrence of asthma) and the day the search was performed (i.e., the 7th of January 2019). Within this observation period, the oldest article investigating a causal relationship between early life antibiotic use and the occurrence of asthma and indexed in PubMed was published in June 1998. The most recent article was published in August 2018. After the exclusion of 3910 articles (explained in detail in figure 1), 63 articles were included (see Appendix 1).

2.4.2 Critical review of the extracted information

Detailed extracted information (cfr. table 1) for all 63 articles can be consulted in Appendix 2. Examples are provided in Appendix 3 to explain how the extracted information was reviewed.

Figure 2: *Flowchart for the selection of articles in PubMed for critical review.*



Notes: Flowchart adapted from Liberati A, Altman DG, Tetzlaff J et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med. 2009;6(7):e1000100.20 Copyright © 2009 Liberati et al. Creative Commons Attribution License.

None of the 63 articles reported a research question and only 16 reported a hypothesis. All articles reported the aim(s) of the study. As a consequence the presence of the outcome, the exposure and the domain was assessed from the formulation of the aim(s) (instead of from the formulation of the research question/hypothesis). For 46 articles, the aim(s) comprised the outcome, the exposure and the domain of the study. None of the 63 articles explicitly reported a "theoretical design". Therefore, all articles were assessed on the presence of seven key elements of

"theoretical design" (measure of occurrence, case (event or state) definition, conceptualization (and operationalization) of the exposure, temporal relation between outcome and exposure, confounders and effect modifiers taken into account and the domain of the study).
Thirteen out of 63 articles reported all seven key elements of "theoretical design" questioned. The other 50 articles reported only three to six out of the seven key elements of "theoretical design" (cf. supra). Table 2 shows the absolute number of articles that (implicitly) reported the listed key elements of the "theoretical design".
Thirteen out of 63 articles reported all seven key elements of "theoretical design" questioned.

The other 50 articles reported only three to six out of the seven key elements of "theoretical design" (cf. supra). Table 2 shows the absolute number of articles that (implicitly) reported the listed key elements of the "theoretical design".

Table 2: *Critical review of 63 articles on the presence of seven key elements of "theoretical design".*

	N (total = 63)
Measure of occurrence	45
Case (event or state) definition	59
Conceptualization and operationalization of the exposure	57
Temporal relation between outcome and exposure derivable from aim and/or case (event or state) definition and conceptualization of the exposure	50
Confounders considered	56
Effect modifiers taken into account	24
Domain of the study (implicitly) reported	63

Forty-five out of 63 articles reported the measure of occurrence (prevalence or incidence of asthma). Out of 63 articles, 59 reported a case (event or state) definition. Fifty-seven out of 63 articles reported the conceptualization and operationalization of the exposure. For 50 articles, the temporal relation between the outcome and the

exposure could be derived from the aim or the case (event or state) definition and conceptualization of the exposure. Regarding confounders and effect modifiers, 56 out of 63 articles reported the confounders that were considered and only 24 out of 63 articles reported to have taken effect modifiers into account. All articles reported the domain of the study. However, none of the authors explicitly referred to this as the "domain".

2.4.3 Derivation of the "theoretical design"

For 50 out of 63 articles, the temporal relation between outcome and exposure could be derived from the aim or the case definition and conceptualization of the exposure. For those 50 articles, HB and JW independently formulated a "theoretical design".

For 15 articles, there was consensus that the authors were interested in investigating "current occurrence of asthma as a function of past exposure to antibiotics". For another 24 articles, there was consensus that the authors were interested in investigating the "future occurrence of asthma as a function of current exposure to antibiotics". For the remaining 11 articles, there was initially no consensus on what the "theoretical design" could be. After discussion, consensus on the "theoretical design" was reached between HB and JW. For 10 articles HB agreed that the "theoretical design" was that formulated by JW and for one article JW agreed with the "theoretical design" to be that formulated by HB. The information reported in these articles was often ambiguous or even missing. Formulating a "theoretical design" was therefore not straightforward. For all scenarios, an example is provided in Appendix 4 to explain this in detail.

2.4.4 Comparison based on before/after the publication of STROBE (2007)

Out of 63 articles, 16 were published between 1998 and 2007 and 47 were published between 2008 and 2019.

Between articles published before and after STROBE, no marked differences were observed neither in the reporting of all seven key elements of "theoretical design" questioned (3/16 vs. 10/47, respectively), nor in the reporting of the outcome, exposure and domain in the aim (pre-STROBE: 11/16 vs. post- STROBE: 35/47).

For the reporting of the individual key elements of "theoretical design" (a-g), there were no marked differences in the articles published after the publication of the STROBE statement (i.e., from 2008 onwards), except for reporting the measure of occurrence and the specification of effect modifiers (table 3).

2.5 Discussion

In this critical review, the reporting of a "theoretical design" in (English language) articles published in scientific peer reviewed journals indexed in the PubMed database investigating the relationship between early life antibiotic use and the occurrence of asthma was assessed (within the observation period from June 1998 until the 7th of January 2019). To our knowledge, no methodological reviews on this topic were published before. Some reviews, investigating only some of the elements of "theoretical design" questioned in this review, were published. For example, a review by Pocock et al. pointed out that the selection of confounders and the justification for the selection of confounders was poorly reported in the reviewed articles.[21] In concordance, another study showed that 22% of the articles examined did not report confounders, and that 33% of the articles did not specify the domain of the study.[22]

The results presented in this critical review are based on our own interpretation of the reported information in the articles. The text of the articles was read twice to ensure that no reported information was overlooked. If there was doubt on the reporting of some of the elements questioned, this was discussed (HB and JW).

Table 3: *Comparison articles published pre- and post-STROBE (2007) for reporting the seven key elements of "theoretical design".*

	1998-2007 (n = 16) n (%)	**2008-2019 (n = 47) n (%)**
Measure of occurrence	14 (87)	31 (66)
Case (event or state) definition	14 (87)	45 (96)
Conceptualization and operationalization of the exposure	15 (94)	42 (89)
Temporal relation between outcome and exposure derivable from aim and/or case (event or state) definition and conceptualization of the exposure	12 (75)	38 (81)
Confounders considered	14 (87)	42 (89)
Effect modifiers taken into account	4 (25)	20 (53)
Domain of the study (implicitly) reported	16 (100)	47 (100)

None of the articles explicitly reported a "theoretical design", although the concept of "theoretical/object design" has been clearly and repeatedly defined since decades.[6,9] Even when the "theoretical design" is not explicitly reported, it can be expected that key elements of the "theoretical design" are reported. We assessed to what extent seven of these key elements were explicitly or implicitly reported in 63 articles. In 13 articles, all seven key elements were present. In all other articles at least three key elements were present. One of these seven key elements reported by all articles was the domain of the study. However, none of the articles explicitly referred to the concept of "domain".

Even though none of the articles explicitly referred to the concept of "theoretical design", referral to the concept of "(study) design" would be expected, since it is an essential element (apart from relevance) both for the justification of the conduct of the study for the authors as for the understanding and interpretation of the findings for the reader. However, only half of the articles (n = 31) explicitly referred to this concept. Of these 31 articles, 16 referred to the concept of "(study) design" in the methods section of the article. Because study design starts after the formulation of an appropriate research question or hypothesis, which is then translated into a theoretical design,[6] it can also be expected that a research question or hypothesis is reported. However, none of the articles reported a research question and 16 out of 63 reported a research hypothesis.

The reporting of the key elements of "theoretical design" questioned was never linked to the concept of "theoretical design". The extraction of the information mentioned in table 1 was not straightforward as this information (including the seven key elements of "theoretical design" questioned) was in most articles unstructured, unclear or even missing. The aim(s) of the study should be stated at the end of the introduction section, preferably followed by the formulation of an appropriate research question or hypothesis. The first paragraph of the methods section should repeat (or formulate) the research question or hypothesis and include the translation hereof into an explicitly formulated "theoretical design". This "theoretical design" should explicitly specify the occurrence relation and the domain of the study. The remaining paragraphs in the methods section should then be dedicated to detailed definition and description of the key elements of "theoretical design" questioned in this critical review (measure of occurrence, case (event or state) definition, conceptualization and operationalization of the exposure, temporal relation between outcome and exposure, confounders that were considered, effect modifiers that were taken into account and

the domain of the study), the design of data collection and the design of data processing.

Concerning the directionality of explanatory research, two opinions within explanatory research exist. One approaches the "theoretical design" in explanatory research as "the future occurrence of the outcome as a function of current exposure in a specific domain", which is inspired by intervention research. This design specifically aims at controlling confounding at scientific reference time T_0 (i.e., moment of realization of the exposure) by making prognostic profiles comparable to each other. The other approaches "theoretical design" in explanatory research as "the current occurrence of an outcome as a function of past exposure in a specific domain", which is inspired by observational research (case-control studies). This approach aims at controlling confounding throughout the whole trajectory prior to the onset of the event or the state (scientific reference time T_0) by reconstructing exposures and other relevant characteristics prior to T_0. This approach has been theoretically elaborated by Miettinen since the eighties of last century: the "object design" ("theoretical design") of explanatory research should be the current occurrence of an outcome as a function of past exposure to a determinant, taking into account relevant covariates (extraneous to the causal pathway: confounders, non-extraneous to the causal pathway: effect modifiers) in a specific domain.[23–26]

Conceptualizing and delineating the "theoretical design" of a study should include the specification of the directionality of the research in the occurrence relation. This is essential for the choice of an appropriate method of data collection, method of data processing and the interpretation of the findings of a study. When the interest is in studying the "current occurrence of an outcome (a state or an event) as a function of past exposure", the scientific reference time T_0 would be at the moment of occurrence of the outcome. When collecting data, the occurrence of the outcome will be identified in the study base and a probe of population time is drawn from the study base. Data on exposure and other relevant characteristics prior to the occurrence of the outcome or selection as a probe of population time must be collected. On the other hand, when the interest is in studying the "future occurrence of an outcome as a function of current exposure profile", scientific reference time T_0 would be at realization (or at the moment of the assessment) of the exposure. The (future) occurrence of the outcome under study would then be compared in the group of exposed and non- exposed (taking into account confounders and modifiers at the moment of the exposure assessment). The data collection method and as a consequence the nature of the data

(exposed can become unexposed) thus depends on the chosen “theoretical design”. Therefore, conflicting findings may (at least partly) be explained by a different underlying “theoretical design” or a mismatch between the “theoretical design” and the design of data collection. Delineating the directionality of explanatory research is essential and explicit reporting of this directionality as part of the occurrence relation of the “theoretical design” would increase quality and transparency of explanatory research. Although the concept of directionality is important, none of the articles reviewed explicitly reported the directionality of the research.

In this critical appraisal, we did not assess to what extent a difference in “theoretical designs” or a mismatch between “theoretical design” and design of data collection would contribute to conflicting results. We propose that future reviews should take this into account.

All (English language) articles published in scientific peer-reviewed journals indexed in the PubMed database and investigating the relationship between early life antibiotic use and the occurrence of asthma were included, resulting in a time period from 1998 to 2019. This allowed to assess reporting based on the year of publication (pre- and post- STROBE). The reporting of key elements of “theoretical design” could also be assessed in articles published before STROBE, because the concept of “theoretical design” is not new and has been introduced early, even before the publication of the oldest article included in this review.

No marked differences were observed, when assessing to what extent the reporting of the seven key elements of “theoretical design” questioned differed after the publication of STROBE. The proportion of articles reporting a measure of occurrence was even smaller post-STROBE compared to pre-STROBE. Although the authors of STROBE advise the reporting of the outcome, the exposure and the domain of the study in the research question[11] (note that in this critical appraisal the presence of outcome, exposure and the domain was assessed in the aim(s) because none of the articles reported a research question), no difference in reporting was observed after STROBE. STROBE was published as a response to the need for reporting guidelines for observational studies.[11] Although STROBE advises the reporting of “key elements of study design”, no specification of what these elements are is provided and no explicit referral to the concept of “theoretical design” is made. Moreover, none of the articles included in this review and published after 2007 reported to have used STROBE as a reporting guideline. In total, according to the Web of Science, STROBE was cited

approximately 16,555 times over a period of almost 13 years.[27] As STROBE was published as a response to the need for reporting guidelines for observational studies, it would be expected that authors would use this guideline in order to improve reporting and therefore would refer to STROBE to support their methodological approach.

When using MeSH terms in PubMed, a selection of articles is made, showing articles that were indexed with these specific terms. Depending on what terms were entered in the PubMed search engine, only a selection is made of all articles indexed in the database and the more specific the terms, the more tailored the search result. To get a broad picture of the current practice in the use of "theoretical design" we kept the search strategy very broad. We only used "asthma" AND "antibiotics" as MesH terms in the PubMed search engine, which resulted in the highest possible number of articles. Neither the domain nor the term "theoretical design" was included as MeSH term, because this would have led to a more detailed selection of articles that were also indexed with these terms and other articles investigating a relationship between early life antibiotic use and the occurrence of asthma would have been overlooked.
We are aware that articles must undergo a reviewing process before publication. As a consequence of the reviewing process, changes could have been made to the first manuscript. This process could have influenced the reporting of the elements questioned in our review. Therefore, we did not intend to draw conclusions about the knowledge of the authors of the articles. We merely wanted to gain insight into the reporting in the final product resulting from this process, which is the published article.

The aim of this critical review was to gain factual knowledge in the reporting of "theoretical design" in (English language) articles published in scientific peer reviewed journals indexed in the PubMed database investigating the relationship between early life antibiotic use and the occurrence of asthma in a well-defined (20.5 years) time period and to assess whether differences in reporting can be observed before and after the publication of STROBE. Therefore, we considered the application of inferential statistics redundant. Nevertheless, it would be instructive to assess reporting in other areas of research assessing a causal relationship between an outcome and an exposure in order to assess whether the same findings can be observed when other outcome-exposure relationships are considered.

2.6 Conclusion

This critical appraisal of research on early life exposure to antibiotics and the occurrence of asthma demonstrated that reporting of the seven key elements of "theoretical design" questioned is still incomplete in explanatory research on the association between early life antibiotic use and the occurrence of asthma. None of the articles reported a "theoretical design" and only one-fourth reported the seven key elements of "theoretical design". No marked differences were observed in the reporting of the seven key elements of "theoretical design" after the publication of STROBE.

Although guidelines do not advise to report an explicit "theoretical design", they do specify important elements of "theoretical design" and how they should be reported (early in the methods section and as specific as possible). "Theoretical design" is a crucial part of study design, setting the scene for the "design of data collection" and the "design of data processing", which is on its turn the backbone for the interpretation of the findings. Conceptualizing, delineating and reporting of "theoretical design" would increase the quality and transparency of explanatory research. This would allow researchers to choose the appropriate method of data collection and method of data processing and would facilitate accurate reporting about their study. Additionally, reporting the "theoretical design" would allow other researchers to reflect on and discuss the quality of the study and what the added value is in the area of research in a more informed way.

2.7 Abbreviations

GINA, Global Initiative for Asthma; IIS, The Infant Immune Study; MD, medical diagnosis; MeSH, medical subject headings; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RERP, Responsible Epidemiological Research Practice; STROBE, Strengthening the Reporting of Observational studies in Epidemiology.

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Chapter 3: Knowledge, Perceptions and Reporting Practices of Theoretical Design in Causal Observational Epidemiological Studies on the Role of Antibiotic Use in the Occurrence of Asthma in Children

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3.1 Abstract

Good research is driven by study design encompassing theoretical design, design of data collection and design of data processing. In epidemiological research, theoretical design is based on a functional relationship between the occurrence and determinants studied (occurrence function) and should also define that part of the theoretical population and the context to which the results pertain (domain). Both are essential for the design of data collection, the design of data processing and the interpretation of the study results and should be explicitly reported. In order to gain insight into the role of theoretical design in the entire research process before publication, it was decided to informally question the corresponding authors of a selection of 30 articles (20 most recent and 10 less recent) reporting on causal observational epidemiological studies on asthma and early life exposure to antibiotics. The objective was to appraise the perceived knowledgeability of theoretical design among the authors of the selected articles. Fifteen authors responded. Authors were asked to indicate their knowledgeability with the concepts of theoretical design, causal theory, confounding and effect modification on a 5-level Likert scale. Other questions were related to the theoretical design of their study. The vast majority of the authors perceived themselves to be moderately to extremely knowledgeable with confounding and effect modification. Perceived knowledgeability of theoretical design and causal theory was more diverse. When provided with options for an occurrence function, almost all

authors indicated “current occurrence as a function of past exposure” for their study. Nevertheless, half of these authors conducted their study based on “future occurrence as a function of current exposure”. Even though the authors perceive themselves to be knowledgeable with theoretical design, this is not reflected in their articles. Theoretical design should be well known, implemented and explicitly reported.

3.2 Background

Good research is driven by study design, encompassing theoretical design, design of data collection and design of data processing (‘analysis’). The theoretical design of a study is the translation of the research question into a research object. In epidemiological research, the object is designed in order to take into account the directionality of the research question. The key elements of the theoretical design depend on the nature (etiologic, diagnostic, prognostic or intervention related) of the functional relationship.[1,2] For example, the functional relationship for a diagnostic research question would be between current prevalence of the target illness and the current diagnostic profile (“current” referring to the moment of diagnostic probability setting). Key elements of theoretical design are: measure of occurrence, case (event or state) definition, conceptualization (and operationalization) of the exposure, temporal relation between outcome and exposure and confounders and effect modifiers taken into account. For the purpose of statistical management, the functional relationship is then expressed as an occurrence function in mathematical terms. The theoretical design is completed with a prespecified (designed) domain (i.e., that part of the theoretical population for which the results are relevant and the context). The theoretical design is essential (the backbone, the motivation) for the design of data collection, the design of data processing and the interpretation of the study results. It is therefore to be expected that apart from a research question, also the theoretical design is explicitly reported in an article.

Scientific knowledge in medicine is expanded by confronting existing evidence with new empirical evidence. Relevant research is therefore replicated taking into account the eventual weaknesses of previous studies. It is to be expected that studies investigating the same exposure-outcome relationship yield slightly different results. Less expected is that study results are contradicting or that differences between study results are large. Contradicting results are usually explained by biases such as residual confounding, information bias, selection bias,… or by unobserved effect modification. Often, design issues are only presented as a weakness (typically without discussing the potential impact on the study results) when the research is not a randomized-controlled trial (RCT), the recommended design to provide evidence of efficacy of interventions.[3,4] When conducting a RCT is not feasible (for causal research), the current recommendation is that the next best option is to conduct a cohort study.

Eventually, a case-control study can be considered as an alternative, being that a case-control study can be interpreted as an efficient version of a cohort study.[5] This point of view, inspired by interventional causal research, is considered as a paradigm for any causal research whether interventional or observational or whether it is aimed at studying causal aspects of an intervention or aimed at causally explaining the genesis of an illness. However, these two types of causality are different and should lead to a different theoretical design. So far, theoretical design is (to our knowledge) never considered in the discussion of the study results.

In our critical appraisal on the use and reporting of theoretical design in studies on the relationship between asthma occurrence and early life antibiotic use, none of the 63 articles reviewed reported a theoretical design and even key elements of theoretical design were not consistently reported.[6] This underreporting could have several reasons, which could not be traced solely based on the critical appraisal: was the theoretical design conceptualized without reporting it, was it not conceptualized at all or was it conceptualized and reported but as a consequence of reviewers' comments they decided to remove it from the manuscript before publication?

To gain insight into the role of theoretical design in the entire research process leading to the publication of an article, we decided to informally question the corresponding authors of a selection of the reviewed articles in our previous work. The objective was to appraise the perceived knowledgeability of theoretical design among the authors of these articles. From the 53 articles, for which we could formulate a theoretical design,[6] we selected 30 articles (20 most recent and 10 less recent, leaving a gap of approximately 10 years in between). The oldest articles were not selected because of practical reasons (e.g., traceability of the authors,…). The theoretical designs that were assigned in consensus in the previous work,[6] can be consulted online: https://zenodo.org/record/3562255#.YVLhUH2xXIU. Other details concerning the questionnaire can be consulted in Appendix 1.[7–10,14]

3.3 Perception of knowledgeability of the concepts of theoretical design, causal theory, confounding and effect modification among the authors

Fifteen authors filled out the questionnaire. The questionnaire of one author was omitted, because he/she specifically requested not to quote the answers.

Knowledgeability was assessed with a 5-level Likert scale (5: extremely knowledgeable; 4: moderately knowledgeable; 3: somewhat knowledgeable; 2: slightly knowledgeable; 1: not at all knowledgeable). Most authors considered themselves to be moderately to extremely knowledgeable with the concepts of confounding and effect modification,

whereas the perceived knowledgeability of causal theory and theoretical design was more diverse. Based on the sum scores for the perception of knowledgeability of theoretical design and causal theory, four groups can (arbitrarily) be distinguished.

> Group 1: Three authors perceiving themselves to be extremely knowledgeable with the concepts of theoretical design and causal theory, with a sum score of 10.
>
> Group 2: Six authors perceiving themselves to be slightly to moderately knowledgeable with the concepts of theoretical design and causal theory, with a sum score of 6–9.
>
> Group 3: Four authors perceiving themselves to be not at all to slightly knowledgeable with the concept of theoretical design and slightly to moderately knowledgeable with the concept of causal theory, with a sum score of 3–5.
>
> Group 4: One author perceiving him/herself to be not at all knowledgeable with all concepts except for confounding, with a sum score of 2.

The authors were asked to formulate a research question for their study and to translate this research question into a theoretical design (including the occurrence function and the domain). To have an idea about their opinion on the directionality of their (implicit) research question, the authors were also asked to select the occurrence function behind their study from one of the seven presented options: (1) Current prevalence of asthma as a function of past exposure to antibiotics; (2) Current incidence of asthma as a function of past exposure to antibiotics; (3) Current prevalence of asthma as a function of current exposure to antibiotics; (4) Current incidence of asthma as a function of current exposure to antibiotics; (5) Future prevalence of asthma as a function of current exposure to antibiotics; (6) Future incidence of asthma as a function of current exposure to antibiotics and (7) Other (specify). In group 1, only one author formulated a research question (with all three essential elements). The same author was able to translate this research question into a theoretical design. Among the three authors, one selected “current occurrence as a function of past exposure”. The remaining author selected “future occurrence as a function of current exposure”. In group 2, only one author formulated a research question and none were able to formulate a theoretical design for their study. All authors selected “current occurrence as a function of past exposure”. In group 3, only one author formulated a research question and none were able to formulate a theoretical design. All authors selected “current occurrence as a function of past exposure”. The author in group 4 did not formulate a research question nor a theoretical design. The author selected “future occurrence as a function of current exposure”.

3.4 Overall appreciation of the answers of the authors

The anonymized answers of the authors to the questions (per group) can be consulted in Appendix 2. The vast majority of the authors (all except for one) did not formulate an occurrence function. This was not entirely surprising, as more than half of the authors did not formulate a research question. The vast majority (all except two) selected “current occurrence as a function of current/past exposure” from the presented options. However, when comparing this with the theoretical design (occurrence function) deduced from the article based on the reported information, half of these authors conducted their study based on “future occurrence as a function of current exposure”. An explanation for this could be the above mentioned preference to conduct a “cohort study” when conducting a RCT is not feasible. No major differences were observed in the answers to the questions between the groups.

Domain seems not to be a known concept. Apparently this is not a commonly used term. Moreover, several definitions can be found for domain. However, domain is an essential part of the theoretical design. Designing the domain, i.e., defining that part of the theoretical population for which the results are relevant in an as well-defined setting (context), will guide an appropriate selection of the study population (e.g.: in intervention research, the domain refers to the population for which the drug is indicated).

Most authors agreed with the comments of the reviewers and for the few that did not agree, changes made to the manuscript before publication were not related to the reporting of a theoretical design. Also, in all studies but one, an epidemiologist was involved.

3.5 Comment

Even though some of the corresponding authors perceive themselves to be knowledgeable with the concept of theoretical design, this cannot be deduced from what is reported in their article. The existing guidelines, such as “the Strengthening the Reporting of Observational Studies in Epidemiology” (STROBE) and “Responsible Epidemiological Research Practice” (RERP), acknowledge the importance of transparent reporting.[11,12] However, though STROBE requests the reporting of key aspects of study design, the guideline does not address in detail what these key aspects are, probably under the assumption that these are well known among researchers.

In medical science, research is often conducted by health professionals lacking a formal training for conducting scientific research. These professionals might have been introduced to scientific research in some of their courses within their basic training, and obviously this introduction is more than appropriate for the qualifications needed to become a good practitioner. However, in order to become a good researcher conducting high-quality scientific research, an in-depth training is essential.[13] Moreover, it appears that merely the presence of an epidemiologist in the team of researchers does not guarantee the appropriate transparency of the report.

We call upon the epidemiological community to reflect on what we observed. We attempted to get an impression of knowledgeability with a few questions only. Should the researchers' knowledgeability of basic research concepts (such as formulating an appropriate research question, translating this question in an appropriate theoretical design, designing a procedure for data collection matching the theoretical design,…) be investigated in a more formal way? In the Netherlands, training programs for epidemiological researchers are not only accredited by the government but also by the Society for Epidemiology (VvE). Should this example be followed, generalized?

We are convinced that the concepts presented in this commentary should be well known and understood, implemented and explicitly reported by researchers conducting medical scientific research. Beyond that, guidelines for reporting could be more explicit in defining what the key elements of study design are. Appropriate reporting of theoretical design probably will increase the emphasis on this part of study design and without doubt consequently improve the quality of medical scientific research (data collection, data management and reporting).

3.6 Conclusion

There is diversity in how authors perceive themselves to be knowledgeable with the concept of theoretical design. This is not reflected in the answers of the authors, since the vast majority did not formulate an appropriate theoretical design. Almost all authors selected "current occurrence as a function of past exposure" for the directionality of their research question when presented with options. However, half of these authors conducted their study based on "future occurrence as a function of past exposure". The reporting of theoretical design in the articles was not influenced by the peer review process. Changes to the manuscripts were not related to the reporting of theoretical design. Basic epidemiological concepts should be well known among researchers conducting medical scientific epidemiological research. We call on the epidemiological community to reflect on the current practice in causal observational

epidemiologic research and on the importance of theoretical design both in research practice and training.

3.7 Abbreviations

RCT: Randomized controlled trial; EBM: Evidence Based Medicine; STROBE: Strengthening the Reporting of Observational Studies in Epidemiology; RERP: Responsible Epidemiological Research Practice; VvE: Society for Epidemiology

3.8 Acknowledgements

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Chapter 4: Asthma occurrence in children and early life systemic antibiotic use: an incidence density study

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4.1 Abstract

Background: Results of studies evaluating the relationship between asthma occurrence and early life antibiotic use have been conflicting. The aim of this study was to investigate the relationship between occurrence of asthma in children and systemic antibiotic use in the first year of life based on an incidence density study with careful consideration of the temporal aspects of the determinant-outcome relationship.

Methods: We conducted an incidence density study nested in a data collection project with information on 1128 mother-child pairs. Systemic antibiotic use in the first year of life was defined as excessive (≥ 4 courses) vs. non-excessive (< 4 courses) use based on information from weekly diaries. Events (cases) were defined as the first parent-reported occurrence of asthma in a child between 1 and 10 years of age. Population time 'at risk' was probed by sampling population moments (controls). Missing data were imputed. Multiple logistic regression was used to assess the association between current first asthma occurrence (incidence density) and systemic antibiotic use in the first year of life, to evaluate effect modification and adjust for confounding.

Results: Forty-seven first asthma events and 147 population moments were included. Excessive systemic antibiotic use in the first year of life showed more than twice the incidence density of asthma compared to non-excessive use (adjusted IDR [95% CI]:

2.18 [0.98, 4.87], p=0.06). The association was more pronounced in children who have had lower respiratory tract infections (LRTIs) in the first year of life compared to children who had no LRTIs in the first year of life (adjusted IDR [95% CI]: 5.17 [1.19, 22.52] versus 1.49 [0.54, 4.14]).

Conclusions: Excessive use of systemic antibiotics in the first year of life may play a role in the genesis of asthma in children. This effect is modified by the occurrence of LRTIs in the first year of life, with a stronger association observed in children experiencing LRTIs in the first year of life.

4.2 Background

Studies worldwide have found disparities in the prevalence of asthma between countries.[1] It has been suggested that these disparities are more likely explained by environmental exposures than by genetic differences.[2] However, there is still a poor understanding of the mechanisms through which factors that are considered fundamental to the development of asthma act. It is now commonly accepted that asthma develops from the complex interplay between genetic and environmental factors.[3,4] In order to explain the marked differences in the prevalence of asthma between countries, some interesting hypotheses have been proposed. One of the first commonly accepted is the hygiene hypothesis. This hypothesis states that the increase in the occurrence of allergic illnesses (including asthma), can be explained by changes in living conditions leading to a reduction in exposure to microorganisms and infections in early life.[5,6] As antibiotics act as an antimicrobial agent and might play a role in different pathways underlying the hygiene hypothesis, several studies have investigated the potential role of antibiotic use in the occurrence of asthma.[7]

Currently, three pathways through which antibiotics may have an influence on the prevalence of allergic illnesses (including asthma) have been proposed. Firstly, antibiotics may remove the protective effect of bacterial infections that are hypothesized to protect against allergic illnesses by modifying the natural course of these infections.[5,7] Secondly, antibiotics with potential anti-inflammatory effects, such as macrolides, may directly inhibit the type 1 immunity response and trigger the development of allergic illnesses.[7,8] Thirdly, antibiotics may act through their influence on the gut microbiome.[8-10] Animal studies have shown that perinatal exposure to antibiotics impacts the gut microbiome and increases the susceptibility to allergic illnesses through the Th2 model.[9] In some epidemiological studies a dose-effect relationship between asthma occurrence and antibiotic use in the first year of life has been observed.[11,12] In two studies the highest risk for asthma was observed in children receiving four or more courses of antibiotics.[12,13] It has been advocated that excessive antibiotic consumption should be avoided given the potential induction of microbial

resistance and the possible association with health outcomes such as asthma.[3,7,14] However, in several European countries antibiotics are still overprescribed and trends for the consumption of antibiotics in Europe hardly changed since 1997.[15,16]

The true nature of the association between asthma occurrence and antibiotic use however remains unclear. Some studies demonstrated a positive association between antibiotic use in early life and the occurrence of asthma,[17-19] whereas others failed to demonstrate an association.[20-23] It has been investigated whether these conflicting results could be explained by 'protopathic bias' or 'reversed causation'.[22,24-26] To allow for a causal interpretation on a determinant-outcome relationship in etiological research, it is quintessential that the temporal aspects of the determinant(s)-outcome relationship are carefully included in the study design (theoretical design, design of data collection and design of data processing).[27] Consequently, the aim of this study was to investigate the relationship between current occurrence of asthma in children and antecedents of systemic antibiotic use in the first year of life with careful consideration of the temporal aspects of the determinant-outcome relationship.

4.3 Methods

4.3.1 Theoretical design

To answer the research question 'what is the relationship between asthma occurrence in children and antecedents of systemic antibiotic use in the first year of life?' this study aims to estimate current incidence density of first asthma occurrence as a function of antecedents of systemic antibiotic use in the first year of life in the domain 'children' (i.e. persons between birth and puberty). The 'primary endpoint' of this study is the incidence density ratio (IDR) as a measure of the strength of the association. In order to allow for a causal interpretation, sex, parental education, breast feeding for at least 6 months, lower respiratory tract infections (LRTIs) and paracetamol (acetaminophen) use in the first year of life, day-care attendance, environmental tobacco smoke (ETS), parental asthma and atopic dermatitis prior to onset were considered either as modifiers or confounders.

4.3.2 Design of data collection

An incidence density study was set up[28] nested within a data collection project collecting information on 1128 mother-child pairs. The project, the Prospective Study on the Influence of Perinatal factors on the Occurrence of Asthma and Allergies (PIPO), started in the province of Antwerp (Belgium) in 1997.[29,30] In the project 2000 pregnant women were invited to participate in order to include a sufficient number of children in the study (aimed sample size was 1200).

Consequently, an appropriate number of respiratory health outcomes would become available during the observation period for precise parameter estimation in studies assessing the relationship between perinatal exposures and respiratory health outcomes.

Data on demographic characteristics, health status, lifestyle and environmental exposures of the mothers and children were collected during two home visits; one at 5 months of pregnancy and one at 3 months post-partum, bi-annually between birth and 4 years of age and annually between 4 and 10 years of age. The questionnaires were based on the standardized questionnaires of the International Study of Asthma and Allergies in Childhood (ISAAC).[31] However, for asthma definition we did not use the ISAAC questions. During the first year of life, a diary was kept by the parents for weekly registration of respiratory symptoms and names of medications administered to the infant.

4.3.2.1 Sampling

As current incidence density cannot be assessed directly and our aim was to only look at relative current incidence density (IDR), we decided to sample according to the principles of a case-control study redefined as explained by Miettinen.[28] In this sampling approach, cases are included as events whereas controls (population moments) are included for probing population time 'at risk'.[28] We decided to assess current incidence density of a (first) parent-reported asthma occurrence in an observation period of 9 years (between 1 and 10 years of age) in order to sample within the domain of the study and in order to collect a sufficient number of events.

Events (first asthma occurrence):

Information on asthma occurrence was parent-reported and obtained from the bi-annual and annual questionnaires. First asthma occurrence was defined as answering for the first time 'yes' to the question *"Has your child had asthma in the past 6 months* (between 1 and 4 years)*/12 months* (between 4 and 10 years)*?"*.First asthma events under the age of 1 year were not included, since the exposure status could only be completed at 1 year of age (cf. infra). Inclusion of first asthma events under the age of 1 year would also not allow the assessment of exposure prior to occurrence of the first asthma event, because no information on the timing of diagnosis was available. Also, diagnosis of asthma under the age of 1 year is even more complex than at other ages.[32,33] Since the domain of the study is children, and puberty can already start at 10 years,[34] all events occurring between 1 and 10 years of age were included in the study.

Population moments:

Eligible population moments (controls) were children still at risk for developing the event (at each follow-up within the PIPO project). Sampling of population moments

was performed within the same observation period as the period for collecting the events (i.e. between 1 and 10 years of age). This was completed in two stages, first by pooling all eligible population moments in a 'risk set'. This 'risk set' then contained all population moments still 'at risk' for the event within the observation period. Secondly, a three times larger (than the events) sample of population moments was randomly (and unmatched) taken from the 'risk set'.

4.3.2.2 Exposure to systemic antibiotics

Information on systemic antibiotic use (including type of antibiotic) in the first year of life was obtained from the weekly diaries. For all events and population moments, the number (courses) of systemic antibiotics (administered either orally, intravenously or intramuscularly) weekly was counted. Systemic antibiotic use in the first year of life was operationalized in two categories: excessive use (≥ 4 courses) and non-excessive use (< 4 courses). As the duration of a course is more or less one week, receiving one course for at least four weeks was considered as excessive use as well. When no information on the exposure was provided in a certain week, this week was considered as a 'missing week'. In case of missing weeks, the number of missing weeks was assessed and the potential impact on the classification in excessive or non-excessive use of systemic antibiotics was evaluated. When the missing weeks might have led to misclassification for the exposure, the questionnaire at 1 year of age was consulted. In case of discordant information, the event or population moment was excluded from the study.

4.3.2.3 Relevant characteristics

Information on relevant characteristics was obtained from the mother's and father's questionnaires at inclusion, during pregnancy, immediately after birth and the bi-annual and annual questionnaires. The procedure differed between events and population moments.

For both events and population moments, information on sex (biological), parental education and breast feeding for at least 6 months was obtained from the questionnaires in the first year of life. Information on parental asthma was obtained from the mother's and father's questionnaire. Information on LRTIs (defined as having had bronchitis with or without chronic cough and/or pneumonia according to the reporting of the parents) and paracetamol (acetaminophen) use in the first year of life was obtained from the questionnaire at 1 year of age. Information on day-care attendance and atopic dermatitis was obtained from all questionnaires (3 months post-partum, bi-annual and annual) prior to or at onset (for events) or prior to or at sampling (for population moments). Information on ETS, which is a time-varying characteristic, was obtained from all questionnaires (at 3 months post-partum, bi-

annual and annual) prior to or at first asthma diagnosis (for events) or at sampling (for population moments).

4.3.3 Design of data processing

Missing data were imputed by applying Multiple Imputation by Chained Equations (MICE).[35] A more detailed explanation of this procedure can be consulted in the supplementary material (additional file 1).

Crude incidence density ratio (IDR) was calculated based on the odds ratio in a 2x2-table including exposure states in first asthma events and population moments and for inference a 95% confidence interval (CI) was estimated.[28] Interaction terms between the exposure and potential effect modifiers were included in multiple logistic regression models to assess effect modification. For deciding on the inclusion or exclusion of an interaction term, an α-level of 0.20 was used.[36] Adjusted IDRs and 95% CIs were estimated by multiple logistic regression accounting for potential modifiers and adjusting for (actual) confounders. Additionally, all statistical modelling was also performed in the complete cases (without imputation of missing data) and can be consulted in the supplementary material (additional file 2).

All relevant statistical information from the output of the regression models (regression coefficients, standard errors, IDRs and 95%CIs) is presented to facilitate the interpretation of the models.[37]

Statistical modelling was carried out in R version 4.2.2.[38]

4.4 Results

4.4.1 Sampling

Within the data collection project with information on 1128 mother-child pairs, 51 first asthma events were identified and 153 population moments were randomly (i.e. not matched) sampled from the 'risk set'. From all 204 records (first asthma events and population moments), 82 (42.3%) had complete weekly diaries (one complete weekly diary equals 52 weeks of information) and 122 (57.7%) had at least 1 missing week with respect to antibiotic use.

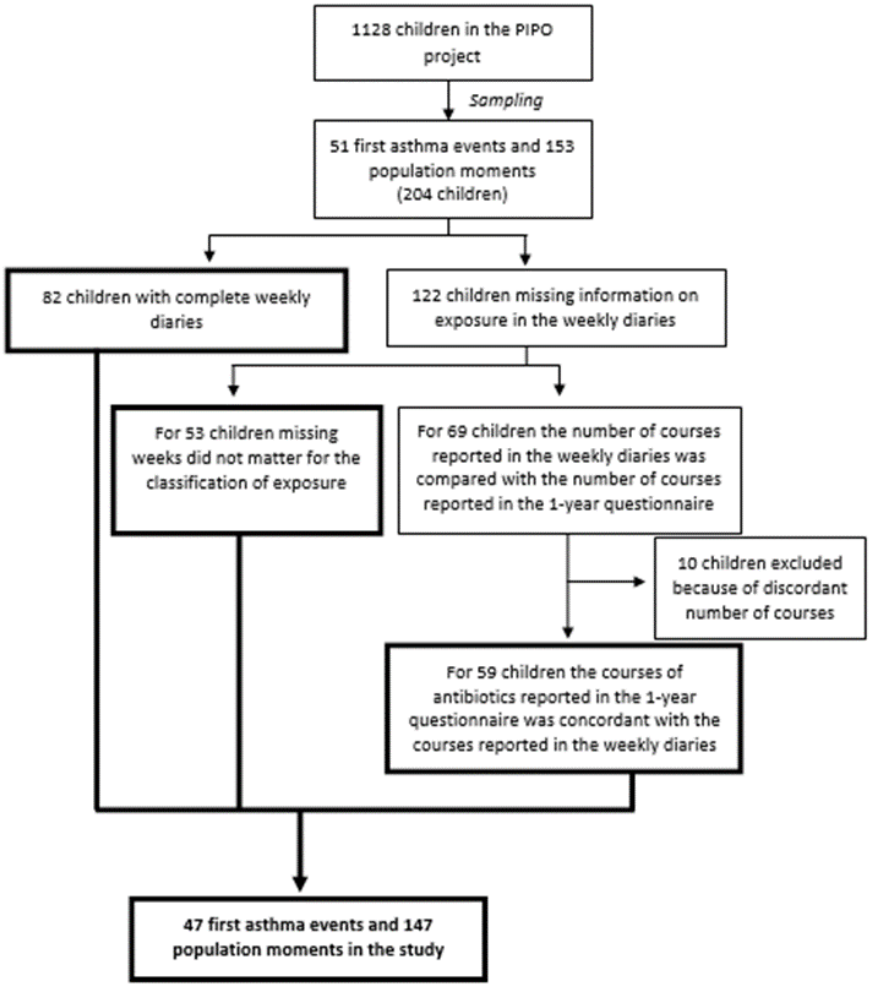

Figure 1: *selection of first asthma events and population moments (controls) taking into account missing exposure in weekly diaries.*

The missing weeks and the possible impact on the classification for the exposure was carefully inspected (figure 1). Forty-seven first asthma events and 147 population moments remained for inclusion in the study.

4.4.2 Relevant characteristics

Table 1 shows the characteristics of the children in whom first asthma events (n = 47) were sampled from ('the events') and in whom population time (n = 147) was sampled from ('the population moments'). The number of courses of systemic antibiotics received in the first year of life ranged from 0 to 13, both in the events and in the population moments.

4.4.3 Missing data on relevant characteristics

There were no missing data for (first) asthma occurrence, exposure and sex. In total, 57 (29.4 %) children in the study had missing data for at least one of the remaining characteristics of interest (17 first asthma events and 40 population moments).

4.4.4 Association between asthma occurrence and antecedents of systemic antibiotic use in the first year of life

4.4.4.1 Crude incidence density ratio (IDR)

In the first asthma events, 14 (29.8%) received four or more courses of systemic antibiotics in the first year of life, whereas in the population moments 28 (19.0%) received four or more courses of systemic antibiotics in the first year of life (table 1). The odds ratio as an estimator for the crude IDR comparing 'excessive exposed' with 'non-excessive exposed' was 1.80 (95% CI 0.85, 3.81).

4.4.4.2 Statistical modelling

The main findings from the multiple logistic regression models using the imputed datasets are presented in table 2. The strength of the association differed with LRTIs in the first year of life, sex, parental education, ETS, parental asthma and atopic dermatitis, but only for 'LRTIs in the first year of life' the estimation of the regression coefficient of the interaction term was precise enough (p-value of the interaction term below the α-level of 0.20) to consider modification. The association between current first asthma occurrence and systemic antibiotic use in the first year of life was more pronounced in children with reported LRTIs in the first year of life (IDR [95% CI] 5.17 [1.19, 22.52]) compared to children with no reported LRTIs in the first year of life (IDR [95% CI] 1.49 [0.54, 4.14]).

In order to allow for comparison with the findings of other studies, the adjusted IDR without interaction term for LRTIs in the first year of life was estimated. Adjusting for confounding by parental education and ETS resulted in an IDR of 2.18 (95% CI 0.98, 4.87). Other characteristics (sex, day-care attendance, breast feeding for at least 6 months and paracetamol (acetaminophen) use in the first year of life) hardly confounded the association, with no changes in the regression coefficient more pronounced than 0.1 decimals.

Table 1: *Characteristics of the children in whom the events and population time were sampled from.*

	First asthma events (n = 47)	Population moments (n = 147)
Age, M*onths, median (min; max)*	48 (18; 108)	42 (18; 108)
Sex, *Female, n (%)*	15 (31.9)	70 (47.6)
Parental education, *High, n (%)*	38 (86.4)	122 (88.4)
Day-care attendance, *Yes, n (%)*	33 (82.5)	118 (82.5)
ETS, *Yes, n (%)*	7 (20.6)	10 (6.8)
Breastfed for at least 6 months, *Yes, n (%)*	10 (22.7)	46 (31.7)
LRTIs first year of life, *Yes, n (%)*	12 (26.1)	39 (26.7)
Paracetamol (acetaminophen) use first year of life, *Yes, n (%)*	40 (87.0)	115 (82.1)
Parental asthma, *Yes, n(%)*	9 (20.5)	22 (15.9)
Atopic dermatitis, *Yes, n(%)*	31 (70.5)	61 (44.9)
Number of systemic antibiotic courses in the first year of life, *n (%)*		
0	20 (42.6)	69 (46.9)
1	6 (12.8)	21 (14.3)
2	4 (8.5)	17 (11.6)
3	3 (6.4)	12 (8.2)
4	3 (6.4)	7 (4.8)
5	3 (6.4)	3 (2.0)
6	3 (6.4)	6 (4.1)
7	1 (2.1)	7 (4.8)
8	1 (2.1)	2 (1.4)
9	1 (2.1)	2 (1.4)
11	1 (2.1)	0 (0.0)
13	1 (2.1)	1 (0.7)
Courses systemic antibiotics in the first year of life, *n(%)*		
Excessive (≥ 4 courses)*	14 (29.8)	28 (19.0)
Non-excessive (< 4 courses)	33 (70.2)	119 (80.9)

*ETS: Environmental tobacco smoke; LRTIs: Lower respiratory tract infections; *IDR = 1.80 (95% CI 0.85, 3.81), $chi^2 = 2.42$, p=0.12*

Table 2: *Results based on crude and adjusted models after multiple imputation of missing data for the association between first asthma occurrence in children and excessive systemic antibiotic use (≥ 4 courses) in the first year of life.*

	B	SE	IDR	95% CI
Crude model				
Excessive systemic antibiotic use in the first year of life	0.59	0.38	1.80	(0.85, 3.81)
Adjusted models				
Excessive systemic antibiotic use[a]	0.78	0.41	2.18	(0.98, 4.87)
Evaluation of effect modification by sex				
Crude model				
Excessive systemic antibiotic use for sex = Male	0.26	0.46	1.29	(0.52, 3.20)
Excessive systemic antibiotic use for sex = Female	1.04	0.69	2.82	(0.72, 10.99)
Interaction term	0.78	0.83†	-	-
Adjusted model[b]				
Excessive systemic antibiotic use for sex = Male	0.44	0.48	1.56	(0.60, 4.03)
Excessive systemic antibiotic use for sex = Female	1.25	0.77	3.49	(0.77, 15.72)
Interaction term	0.81	0.90†	-	-
Evaluation of effect modification by parental education				
Crude model				
Excessive systemic antibiotic use for parental education = Low	0.77	0.97	2.16	(0.32, 14.37)
Excessive systemic antibiotic use for parental education = High	0.56	0.42	1.75	(0.76, 4.01)
Interaction term	-0.21	1.06†	-	-
Adjusted model[c]				
Excessive systemic antibiotic use for parental education = Low	1.38	1.11	3.99	(0.45, 35.42)
Excessive systemic antibiotic use for parental education = High	0.69	0.44	1.99	(0.84, 4.72)
Interaction term	-0.70	1.19†	-	-
Evaluation of effect modification by ETS				
Crude model				
Excessive systemic antibiotic use for ETS = No	0.60	0.43	1.83	(0.78, 4.28)
Excessive systemic antibiotic use for ETS = Yes	1.19	1.26	3.29	(0.28, 38.78)
Interaction term	0.58	1.34†	-	-
Adjusted model[d]				
Excessive systemic antibiotic use for ETS = No	0.69	0.44	1.99	(0.84, 4.73)
Excessive systemic antibiotic use for ETS = Yes	1.53	1.30	4.62	(0.36, 59.46)
Interaction term	0.84	1.38†	-	-
Evaluation of effect modification by LRTIs in the first year of life				
Crude model				
Excessive systemic antibiotic use for LRTIs first year of life = No	0.26	0.50	1.30	(0.49, 3.45)

Excessive systemic antibiotic use for LRTIs first year of life = Yes	1.31	0.69	3.70	(0.96, 14.24)
Interaction term	1.05	0.85	-	-
Adjusted model[e]				
Excessive systemic antibiotic use for LRTIs first year of life = No	0.40	0.52	1.49	(0.54, 4.14)
Excessive systemic antibiotic use for LRTIs first year of life = Yes	1.64	0.75	5.17[*]	(1.19, 22.52)
Interaction term	1.24	0.91[**]	-	-
Evaluation of effect modification by parental asthma				
Crude model				
Excessive systemic antibiotic use for parental asthma = No	0.53	0.41	1.69	(0.75, 3.81)
Excessive systemic antibiotic use for parental asthma = Yes	1.74	1.29	5.71	(0.45, 72.29)
Interaction term	1.21	1.36[†]	-	-
Adjusted model[f]				
Excessive systemic antibiotic use for parental asthma = No	0.78	0.45	2.18	(0.91, 5.27)
Excessive systemic antibiotic use for parental asthma = Yes	2.14	1.38	8.54	(0.56, 128.95)
Interaction term	1.36	1.45[†]	-	-
Evaluation of effect modification by atopic dermatitis				
Crude model				
Excessive systemic antibiotic use for atopic dermatitis = No	0.99	0.63	2.70	(0.78, 9.31)
Excessive systemic antibiotic use for atopic dermatitis = Yes	0.42	0.51	1.52	(0.56, 4.12)
Interaction term	-0.57	0.82[†]	-	-
Adjusted model[g]				
Excessive systemic antibiotic use for atopic dermatitis = No	1.11	0.64	3.04	(0.86, 10.71)
Excessive systemic antibiotic use for atopic dermatitis = Yes	0.64	0.55	1.89	(0.64, 5.54)
Interaction term	-0.47	0.85[†]	-	-

*ETS: Environmental tobacco smoke; LRTIs: Lower respiratory tract infections; β: regression coefficient; SE: standard error; IDR: incidence density ratio; CI: confidence interval; p: p-value; [a]Adjusted for confounding by parental education and ETS; [b]Adjusted for confounding by parental education and ETS and taking into account effect modification by sex; [c]Adjusted for confounding by ETS and taking into account effect modification by parental education; [d]Adjusted for confounding by parental education and taking into account effect modification by ETS; [e]Adjusted for confounding by parental education and ETS and taking into account effect modification by LRTIs in the first year of life; [f]Adjusted for confounding by parental education and ETS and taking into account effect modification by parental asthma; [g]Adjusted for confounding by parental education and ETS and taking into account effect modification by atopic dermatitis; *p<0.05; **p for interaction term <0.20; †p for interaction term > 0.20*

4.5 Discussion

Based on our findings and after adjustment for confounding by parental education and ETS, children exposed to four or more courses of systemic antibiotics in the first year of life seemed to have more than twice the incidence density for asthma occurrence than children exposed to less than four courses (IDR 2.18 (95% CI 0.98, 4.87)). Although our estimation was rather precise (p=0.06) this association is not statistically significant at the predominantly used α-level of 0.05. Similar associations have been reported in previous studies.[39-41] Ni et al. reported in their study that antibiotic use in the first year of life is associated with asthma occurrence in children between 1 and 10 years of age (OR [95% CI] 2.66 [1.11, 6.40]).[40] Su et al. reported in their study that use of systemic antibiotics in the first 9 months of life is associated with asthma in children up until the age of 5 years (adjusted OR: 1.50, p = 0.047).[42]

In the theoretical design, we indicated that the interest within this study was also to investigate possible effect modification by sex, parental education, ETS, LRTIs in the first year of life, parental asthma and atopic dermatitis. For the specified characteristics, interaction terms were added to the model. Deciding on whether or not to include the interaction term in the final model (and therefore whether or not to reject the null hypotheses of no interaction), was based on an α-level of 0.20. For sex, parental education, ETS, parental asthma and atopic dermatitis the interaction term exceeded the α-level of 0.20, limiting the evidence for effect modification. This was not the case for LRTIs in the first year of life. When including the interaction term for LRTIs in the first year of life in the adjusted model (where the association between excessive systemic antibiotic use and the occurrence of asthma was not significant at the α-level of 0.05), the adjusted association appeared to be much stronger in children who have had LRTIs in the first year of life, with a statistically significant IDR of 5.17 (p = 0.03) compared to in children who did not have LRTIs in the first year of life (IDR 1.49).

The observed association between excessive systemic antibiotic use in the first year of life and asthma occurrence in children who have had LRTIs in the first year of life, might be biologically explained by the composition of the lung microbiome.[43,44] Both exposure to antibiotics and viral respiratory tract infections in early life can disrupt the composition of the lung microbiome, leading to an increased risk for the development of asthma.[43,45] It is therefore plausible that the strong association observed in our study in children reporting LRTIs in the first year of life might be explained by a biological interplay between these two exposures. On the other hand children receiving an excessive amount of antibiotics in the first year of life and with LRTIs in the first year of life, might have received the antibiotics for the treatment of recurrent respiratory symptoms that are in fact not related to LRTIs. These recurrent respiratory symptoms in the first year of life might indeed already be an early sign of the presence of an

obstructive pulmonary disease (asthma), implying that some children with this profile are predisposed for a later diagnosis of asthma (misclassification with respect to diagnosis of LRTIs). We assessed whether effect modification by LRTIs in the first year of life would still be observed after exclusion of the subjects reporting pneumonia in the first year of life. After exclusion of these subject (n = 9), effect modification by LRTIs was still observed. We therefore suggest that future studies with larger sample sizes elaborate on the possible biological pathways including the role of LRTIs.

Evidence for an association between exposure to antibiotics in early life and the occurrence of asthma has been controversial. Some studies failed to demonstrate an association between antibiotic use in early life and the occurrence of asthma in children.[20] These conflicting results could be (at least partially) explained by a mismatch between the 'theoretical design' of the study and the design of data collection. In etiological research, when the interest is in studying the *'current occurrence of an event as a function of prior exposure'*, data on exposure and relevant characteristics (modifiers and confounders) must be collected prior to onset of the event or prior to and up to sampling as a population moment. When the interest is in studying the *'future occurrence of an event as a function of current exposure'* (i.e. a prognosis oriented rather than an etiology oriented design), then data on relevant characteristics must be collected at the moment of the realization of the exposure. A mismatch between the theoretical design (etiology oriented, whether or not explicit) and the design of data collection (prognosis oriented), could therefore lead to misinterpretation of study results leading to conflicting study results.[28] In our study, the design of data collection was carefully set up to match with the 'theoretical design'. Our theoretical design was etiology oriented ('current occurrence of asthma as a function of past exposure to systemic antibiotics'). The design of data collection matched with the theoretical design, assessing current incidence density of asthma as a function of prior exposure to systemic antibiotics. All characteristics taken into account were prespecified in the theoretical design and temporality was also taken into account for these characteristics. We believe this is a strength of our study.[46,47]

Another explanation for the diverging results in literature on the relationship between early life antibiotic use and the occurrence of asthma could be the population mix of the different studies and failing to take this population mix (regarding the proportion of children with LRTIs in the first year of life) into account. Depending on the prevalence of LRTIs in a study (high proportion of children with LRTIs vs. low proportion of children with LRTIs in the first year of life), it is more likely that the association between exposure to systemic antibiotics in the first year of life and occurrence of asthma would be observed in studies with a high proportion of children reporting LRTIs in the first year of life. Therefore, we advise further studies on the relationship between antibiotic use and occurrence of asthma in children to take LRTIs into account.

Our study has several weaknesses. The number of events in our study was low, which is reflected in the width of the confidence intervals for the association in children reporting LRTIs in the first year of life. Therefore, the results need to be interpreted with caution.

Asthma is a complex clinical disease difficult to diagnose under the age of 6.[48,49] According to a recent study by Yang et al., childhood asthma is often misdiagnosed.[50] In our study, 37 first asthma events under the age of 6 were included. This implies that some of the first asthma events could be misdiagnoses, but also that asthmatic children with respiratory symptoms in early life could have been missed and therefore included as population moments in our study. This problem of misclassification may have led to biased results. Moreover, if studies adjust for confounding by respiratory diagnoses (e.g. LRTIs) under the age of 6, overadjustment of the studied association might be a consequence if the respiratory diagnoses adjusted for are misdiagnoses. Being aware of this possibility of misclassification, we did include first asthma events under the age of 6 in our study, because we assumed that the antibiotic use in early life is probably more relevant for the events identified already in early life. For 13 out of the 37 first asthma events under the age of 6 the parents reported that the child suffered from asthma after the age of 6. We additionally assessed whether the other 24 first asthma events under the age of 6 reported other symptoms that could be typical for asthma. For 17 out of the 24 first asthma events wheezing and/or shortness of breath occurred at first asthma occurrence and was reported in at least one subsequent questionnaire. For the remaining 7 first asthma events, asthma was only reported in one questionnaire and symptoms were not persistent during the entire observation period, implying that these asthma events might be misclassified. We also checked for all asthma events whether asthma medication was used at the same time. For 40 out of 47 first asthma events, asthma medication was used at the moment of diagnosis. The other 7 first asthma events did not use asthma medication, however we decided to include them in our study because non-medicinal control of asthma is also possible (e.g. avoidance of exposures).

We did not look at antibiotic subtypes nor dose-effect, which might be important for the biological interpretation of our findings. Some studies investigated the relationship between exposure to subtypes of antibiotics and asthma in childhood. Örtqvist et al. reported in their study that the risk of asthma was more pronounced for exposure to antibiotics for the treatment of respiratory infections (HR [95% CI] 4.12 [3.78, 4.50]) compared to antibiotics for the treatment of urinary tract and skin infections (HR [95% CI] 1.54 [1.24, 1.92]).[51] Unfortunately, the number of events in our study was too small to look at subtypes of antibiotics or dose-effect. We did not consider use of antibiotics during pregnancy, since the aim was limited to investigating the effect of exposure in the first year of life. Antibiotic use during childbirth was not considered, because no data on this exposure were available.

Despite the weaknesses, our study has several strengths. One of the strengths of our study is that the data used were collected in a project that was specifically designed to determine pre- and postnatal risk factors for childhood asthma. In the first year of life detailed information on antibiotic use was collected in weekly diaries. In most studies, such frequent collection of exposure information is rare and usually subjected to recall bias. In our study, the possibility of recall bias and misclassification for the exposure is minimized. Also, the collection of detailed information during the observation period allowed us to assess all relevant characteristics in accordance with the theoretical design of our study.

The probing of population time by (unmatched) sampling of population moments from a 'risk set' is an additional strength. If the population moments would have been sampled in the same databases the events were identified in, this would have led to matching on age which could have introduced bias.[52] Moreover, the procedure applied for sampling population moments allowed for including population moments in the study that were still 'at risk' for the event at the moment of sampling, but could have become an event later in time. Using this approach limits the risk for selection bias.

If the information on the exposure from the weekly diaries was inappropriate to allow for classification for the exposure status (as a consequence of missing weeks), the event or population moments was excluded from the study. Also, missing data were imputed using MICE. Most studies on this topic are often based on complete cases only. These results can be biased if selection bias is introduced because of missingness. We did also perform the statistical modelling with the complete cases only (see additional file 2). The associations were stronger in the complete case analysis, but the confidence intervals were wider.

4.6 Conclusion

In conclusion, the observed associations in our study indicate a relationship between the occurrence of parent-reported asthma in children and excessive systemic antibiotic use in the first year of life. The association was more pronounced (and statistically significant at the α-level of 0.05) in children who have had LRTIs in the first year of life. We therefore suggest to conduct further studies to assess in more detail the nature of this relationship between the occurrence of asthma and excessive systemic antibiotic use with special attention for the subtypes of antibiotics and dose-effect, focusing on the possible role of LRTIs in early life. Meta-analysis of existing studies or grouping data from different birth cohort projects could be a valuable basis for further research.

4.7 List of abbreviations

CI: Confidence interval; ETS: Environmental tobacco smoke; IDR: Incidence density ratio; ISAAC: International Study of Asthma and Allergies in Childhood; LRTIs: Lower respiratory tract infections; MICE: Multiple imputation by chained equations; PIPO: Prospective Study on the Influence of Perinatal Factors on the Occurrence of Asthma and Allergies

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Chapter 5: The Association between the Occurrence of Asthma and Antecedents of Exposure to Environmental Tobacco Smoke in the Previous Year in Children: An Incidence-Density Study

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5.1 Abstract

In previous studies, the strength of the association between childhood asthma and environmental tobacco smoke (ETS) differed depending on the way ETS was assessed and the type of study conducted. We investigated the relationship between asthma occurrence in children and recent exposure to ETS based on an incidence-density study driven by the explicit formulation of a theoretical design. Additionally, we assessed whether the relationship is modified by perinatal ETS exposure and parental inhalation atopy. The event was conceptualized as 'first doctor's diagnosis of asthma'. Population time was probed by sampling population moments. Exposure to ETS was conceptualized as recent exposure (1 year prior to diagnosis or at sampling) and perinatal exposure (in utero and/or during the first year of life). Thirty-nine events and 117 population moments were included. There was no indication for effect modification by perinatal exposure to ETS or parental inhalation atopy. After adjustment for confounding, an association was observed between occurrence of a first asthma diagnosis and recent ETS exposure: incidence-density ratio 4.94 (95% confidence interval 1.21, 20.13). Asthma occurrence in children is associated with recent exposure to ETS, and this association seems not to be modified by perinatal ETS exposure or parental inhalation atopy.

5.2 Introduction

According to the Global Burden of Disease (GBD) study, asthma affected approximately 262 million people and caused 461,000 deaths in 2019 [1,2]. Among children, asthma is the most common chronic disease [1]. Asthma is a heterogeneous disease which is (usually) characterized by chronic airway inflammation and defined by a history of respiratory symptoms (wheezing, shortness of breath, chest tightness and cough). These symptoms can vary over time and in intensity, and occur together with variable expiratory airflow limitation [3]. The underlying causal mechanisms in the development of asthma are still not completely understood. However, there is general consensus among experts that the inception and persistence of asthma is influenced by gene-environment interactions and that a 'window of opportunity' exists during the perinatal period (i.e., in utero and during the first year of life) when the immune system is still developing and environmental risk factors have the opportunity to influence this development, and as a consequence may influence the onset of asthma [3].

Based on insights from in vitro studies and observational studies, the current advice for the prevention of asthma is to avoid exposure to ETS during pregnancy and in the first year of life [3]. In a systematic review and meta-analysis of cohort studies investigating the effect of smoking by parents or household members on the risk of asthma, Burke et al. reported that in young children (aged ≤ 2 years) the strongest effect was observed for in utero exposure to ETS (through active smoking of the mother). This effect weakened with increasing age, but remained present. Postnatal exposure to ETS, on the other hand, was only associated with the incidence of asthma in older children (aged 3-18 years) [4]. In a recent systematic review and meta-analysis by He et al., it was shown that exposure to ETS (for any exposure time window) was associated with a higher risk of a doctor's diagnosis of asthma in children [5]. However, differences in the strength of the associations were observed depending on the way ETS was conceptualized (in utero exposure vs. postnatal exposure). In utero ETS exposure was more strongly associated with asthma occurrence than postnatal ETS exposure [5]. In a subgroup analysis, He et al. divided the studies included according to type (case-control studies/cross-sectional studies vs. cohort studies). In case-control studies and cross-sectional studies, in utero ETS exposure was more strongly associated with asthma occurrence than postnatal ETS exposure, while in cohort studies this was the other way around [5].

Several biological mechanisms can explain the relationship between exposure to ETS and the occurrence of asthma. Two indirect effects are the modification of the

development of the immune system by suppressing the Th1 immune response and enhancing the Th2 immune response, and the modulation of the ubiquitin-proteasome pathway [6,7]. The toxicity of the chemicals in tobacco smoke can also directly affect the respiratory epithelium and smooth muscle tissues of the lungs [6,7]. Even though the relationship between exposure to ETS and the occurrence of asthma in children has been investigated extensively, only a few studies operationalized exposure to ETS in several ways (both in utero exposure and postnatal exposure) [8-11]. However, and to the best of our knowledge, none of these studies investigated the relationship between a doctor's diagnosis of asthma with recent exposure to ETS (outside the perinatal period but prior to asthma onset), even though it is hypothesized that ETS exposure can also have direct effects on the lungs. Therefore, the aim of this study is to investigate the relationship between current asthma occurrence in children and antecedents of recent ETS exposure by conducting an incidence-density study. Additionally, we assessed whether the relationship between current asthma occurrence in children and recent ETS exposure is modified by perinatal exposure to ETS and parental inhalation atopy.

5.3 Materials and Methods

5.3.1 Research Question and Theoretical Design

The aim of the study led to the following research question: What is the relationship between asthma occurrence in children and antecedents of recent exposure to ETS (1 year prior to diagnosis), and is this relationship modified by perinatal exposure to ETS and parental inhalation atopy?

In order to be able to answer the research question, a theoretical design was formulated: current incidence (density) of asthma in children as a function of prior exposure (1 year prior) to ETS taking into account effect modification by perinatal exposure to ETS and parental inhalation atopy and adjusting for confounding by age, sex, parental education and day-care attendance, and in case of no indication for effect modification also for perinatal exposure to ETS and parental inhalation atopy.

The domain is children prior to puberty.

A directed acyclic graph (DAG) was constructed to determine what covariates to adjust for.

5.3.2 Design of Data Collection

Data were collected in the Prospective data collection project on the Influence of Perinatal factors on the Occurrence of Asthma and Allergies (PIPO) [12,13]. This project started in 1997 in the province of Antwerp, including 1128 children and collecting information on the mothers (pregnancy) and offspring in order to investigate the perinatal risk factors for the occurrence of asthma and other allergic illnesses in childhood. Information on outcome, exposure and relevant characteristics was obtained from the mother's and father's questionnaires, the questionnaire at the second home visit, the bi-annual questionnaires between birth and 4 years of age and the annual questionnaires between 4 and 8 years of age.

We defined current incidence density as the number of events (cfr. infra) divided by the summation of all observation periods 'at risk' for the event in an observed population, for an observation period going to zero (instantaneous incidence density). It is clear, however, that the assessment of instantaneous incidence density is not feasible, as the measurement of exposed and unexposed population time becomes unfeasible when the observation period actually goes to zero. Therefore, a quasi-incidence density sampling over a non-zero observation period was set up [14].

5.3.2.1 Sampling

Quasi-incidence density of asthma was assessed in an observation period of 7 years (between 1 and 8 years of age) in order to cover the domain and to collect a sufficient number of events.

Events

Events were conceptualized as parent-reported first doctor's diagnoses of asthma. A parent-reported first doctor's diagnosis of asthma was defined as answering for the first time 'yes' to the question "Was your child suffering from asthma in the previous six months and was this confirmed by a doctor?" (between 1 and 4 years) or "Was your child suffering from asthma in the previous 12 months and was this confirmed by a doctor?" (between 4 and 8 years of age). Events were therefore first doctor's diagnoses of asthma between the age of 1 and 8 years. Events under the age of 1 year were excluded from the study.

Population Time

As measuring the entire population time in a dynamic exposure experience is almost impossible and not essential for the valid estimation of the incidence-density ratio,[14] we decided to probe population time in two stages. First, all records (at each follow-

up) from the PIPO project leading to the collection of information were considered as a probe for the evolving population time in the participating children in the project. We refer to these as population moments. At each follow-up within the PIPO project, all population moments still 'at risk' for developing the event were entered in a 'risk set'. This 'risk set' contained all population moments still 'at risk' for the event within the same observation period as the period for collecting the events (between 1 and 8 years of age). Secondly, from this 'risk set', a random and unmatched sample of population moments was taken as a probe of the study base, and after merging with the events, extensive information on the sampled records (events and population moments) was retrieved.

5.3.2.2 Recent Exposure to Environmental Tobacco Smoke

Recent exposure to ETS was defined as exposure to ETS 1 year prior to the first doctor's diagnosis of asthma or sampling of the population moment. This information was retrieved from the bi-annual and annual questionnaires in which the parents were asked the following question: In the last 6 months/12 months, was your child regularly exposed to tobacco smoke (cigarettes, cigars, pipe)? ('Regularly' is most days of the week.)

All exposure variables were dichotomized into 'exposed' vs. 'unexposed'.

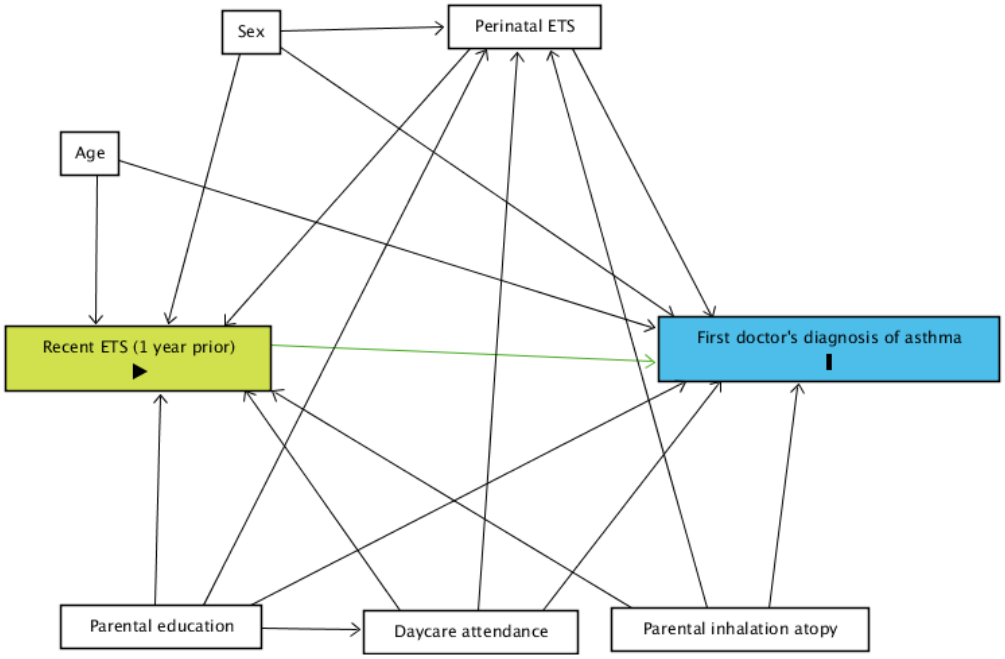
5.3.2.3 Relevant Characteristics

A directed acyclic graph (DAG) was constructed using 'DAGitty v.3.0' for the relationship between recent exposure to ETS and asthma diagnosis (Figure 1). [15]

Perinatal exposure to ETS (in utero exposure of the child to ETS and/or exposure to ETS during the first year of life, yes vs. no) and parental inhalation atopy (self-reported hay fever, yes vs. no) were considered as potential modifiers.

Age (in months), sex (male vs. female), parental education (high vs. low) and ever attending daycare were considered as potential confounders. In case of no indication for effect modification, perinatal exposure to ETS and parental inhalation atopy were considered as potential confounders as well.

Figure 1: *DAG for the relationship between the current occurrence of a first doctor's diagnosis of asthma and recent exposure to ETS. Green: exposure; blue: event; ETS: environmental tobacco smoke.*



5.3.3 Design of Data Processing

5.3.3.1 Handling of Missing Data

Missing data were imputed by applying Multiple Imputation by Chained Equations (MICE).[16] In the imputation model, all variables of interest for the model (cfr. supra) were included. The number of imputed datasets was set at 30 and the logistic regression method was used for imputation.

5.3.3.2 Statistical Modelling

Data were summarized in a 2 × 2 table and odds ratios (ORs) were calculated for the estimation of the crude (quasi-) incidence-density ratios (IDRs) with estimation of 95% confidence intervals (CI). As the IDRs were estimated by calculating ORs, multiple logistic regression was applied to assess the presence of effect modification and to control for confounding. An α-level of 0.20 was used to decide on the inclusion of an interaction term.

In order to facilitate the interpretation of the regression models, all relevant statistics are presented (regression coefficients, standard errors, IDRs and 95% CIs). [17] All statistical procedures were performed in R version 1.4.1106.[18]

5.3.4 Ethics Approval

The medical ethics committee of the University Hospital of Antwerp granted approval for all parts of the project (UA A06 10) and informed written consent was obtained from the parents for each assessment during the project.

5.4 Results

5.4.1 Sampling

In total, 39 events (first doctor's diagnoses of asthma between 1 and 8 years of age) were identified. A sample of 117 unmatched population moments was randomly taken from the 'risk set'. In total, the number of records was 156.

5.4.2 Relevant Characteristics

Table 1 shows the characteristics of the events (n = 39) and the population moments sampled as a probe of population time (n = 117). In the events, the proportion of females was smaller compared to the population moments. Events were more often exposed to ETS in the year prior to diagnosis and perinatally compared to the population moments. A higher proportion of events had parents with inhalation atopy compared to the population moments.

5.4.3 Missing Data

There were no missing data for the outcome (first doctor's diagnosis of asthma), age and sex. In total, 27 (17.3%) records had at least one missing value for the remaining characteristics (recent ETS exposure, perinatal ETS exposure, parental education, day-care attendance and parental inhalation atopy).

Table 1: *Characteristics of the children in whom the events (first doctor's diagnoses of asthma, n = 39) were sampled from and population time was probed from (population moments, n = 117).*

	Events (n = 39)	Population Moments (n = 117)
Age, months, median (IQR)	48 (30)	42 (42)
Sex, female, n (%)	10 (25.6)	65 (55.6)
Parental education, high, n (%)	31 (86.1)	103 (90.4)
Daycare attendance, yes, n (%)	27 (79.4)	95 (84.1)
Parental inhalation atopy, yes, n (%)	25 (71.4)	53 (46.1)
Perinatal ETS, yes, n (%)	17 (48.6)	29 (25.9)
Recent ETS exposure, yes, n (%)	6 (15.4)	8 (6.8)

IQR: interquartile range; ETS: environmental tobacco smoke.

5.4.4 Relationship between Current Occurrence of First Doctor's Diagnosis of Asthma and Recent Exposure to ETS

5.4.4.1 Crude Incidence-Density Ratios

In the crude analysis, an association was observed between the occurrence (incidence density) of a first doctor's diagnosis of asthma and recent exposure to ETS: IDR 2.62 (95%CI 0.92, 7.52).

5.4.4.2 Effect Modification by Perinatal ETS Exposure and Parental Inhalation Atopy

We were not able to assess effect modification by parental inhalation atopy because of empty cells. We found no evidence for effect modification by perinatal exposure to ETS because the estimation of the regression coefficient of the interaction terms exceeded the α-level of 0.20 (Table 2).

Table 2: *evaluation of effect modification of the relationship between the occurrence of a first doctor's diagnosis of asthma and recent exposure to ETS by perinatal exposure to ETS.*

	β	SE	IDR	95% CI
Recent exposure to ETS for perinatal exposure to ETS = unexposed	0.78	1.25	2.18	0.19–25.31
Recent exposure to ETS for perinatal exposure to ETS = exposed	0.42	0.65	1.52	0.42–5.49
Interaction term	−0.36	1.42 §	-	-

ETS: environmental tobacco smoke; §: p > 0.20.

5.4.4.3 Adjustment for Confounding

After adjustment for confounding by age, sex, parental education, perinatal exposure to ETS, day-care attendance and parental inhalation atopy (Table 3), the association observed between the occurrence of a first doctor's diagnosis of asthma and recent exposure to ETS was: IDR 4.94 (95% CI 1.21, 20.13).

Table 3: *Crude and adjusted associations for the relationship between the occurrence of a first doctor's diagnosis of asthma and recent exposure to ETS.*

	β	SE	IDR	95% CI
Recent exposure to ETS (crude)	0.96	0.54	2.62	(0.92, 7.52)
Recent exposure to ETS (adjusted)*	1.60	0.72	4.94 †	(1.21, 20.13)
Constant	−2.12	0.98	0.12 †	(0.02, 0.81)
Age	0.01	0.01	1.01	(0.99, 1.02)
Sex	−1.48	0.49	0.23 †	(0.09, 0.59)
Parental education	0.38	0.78	1.47	(0.32, 6.82)
Daycare attendance	−0.46	0.57	0.63	(0.21, 1.95)
Perinatal exposure to ETS	0.80	0.48	2.22	(0.87, 5.67)
Parental inhalation atopy	1.16	0.48	3.18 †	(1.23, 8.20)

*ETS: environmental tobacco smoke; * Adjusted for confounding by age, sex, parental education, day-care attendance, perinatal exposure to ETS and parental inhalation atopy; †p < 0.05.*

5.5 Discussion

In this study, we observed a strong (IDR 4.94 [95% CI 1.21, 20.13]) association between the occurrence of a first doctor's diagnosis of asthma and recent exposure to ETS (1 year prior to diagnosis). These findings imply that exposure in early childhood, also outside of the perinatal window, is associated with the occurrence of asthma. Other studies mainly focused on in utero exposure to ETS and/or exposure in early life (future occurrence as a function of current exposure instead of current occurrence as a function of past exposure) [10,19].

Additionally, we evaluated whether the association was modified by perinatal exposure to ETS. We did not find an indication for effect modification by perinatal ETS exposure.

Even though we observed a strong and statistically significant association (α-level 0.05) between the occurrence of a first doctor's diagnosis of asthma and recent exposure to ETS, the sample size of our study is rather small. We were only able to identify 39 first doctor's diagnoses of asthma. Therefore, we are aware that the results of this study should be interpreted with caution.

Some studies assessed ETS exposure and the occurrence of asthma cross-sectionally and found an association between ETS exposure and asthma occurrence [20,21]. However, in cross-sectional studies the strength of the association might be underestimated due to misclassification of the exposure. Parents of children experiencing asthmatic symptoms might stop exposure of their child to ETS early on. At the same time, recall bias and social desirability bias might lead to misclassification of the exposure in cross-sectional studies. Our study is (to the best of our knowledge) the first in which exposure to ETS is reconstructed as an antecedent 1 year prior to asthma diagnosis. Our findings are supported by previous findings from studies investigating the effects of exposure to ETS on markers of inflammation on a molecular biological level.

Our findings confirm the hypothesis proposed in studies on a molecular biological level assessing the direct effects of ETS on pathways of inflammation: that is, apart from being involved in the development of the immune system, ETS can also have direct inflammatory effects and act as a trigger [22].

In several studies, it was observed that healthy children exposed to ETS had higher levels of serum IL-4, IL-5, total IgE and a higher absolute number of blood eosinophils and also elevated levels of TNF-α and INF-γ [23–25]. In another study, elevated IL-5, IL-6, TNF-α and INF-γ after 1 h were also observed in healthy adult non-smokers that were exposed to tobacco smoke [26].

Asthma is a complex disease, and diagnosing it, especially in young children, is challenging [3]. In our study, we only included first doctor's diagnoses of asthma, but we are aware that this could have led to the missing of events. However, we preferred this type of misclassification as defining the event based on parent-reported symptoms would have inflated the number of events by observations that are probably not related to ETS (but to infections). This would have led to an underestimation of the true effect of ETS. For the same reason, we did not include events under the age of 1 year, because in these cases, wheezing and other respiratory symptoms occur very frequently (e.g., as a consequence of mild viral respiratory tract infections), making a diagnosis of asthma even more difficult [27].

The main exposure (recent exposure to ETS) was assessed 1 year prior to diagnosis of asthma. We did not take into account the duration of the exposure or the dose, although Chau-Etchepare et al. pointed out that this might be important to consider [22]. We did not consider the duration of exposure because we would in some cases not be able to distinguish between perinatal exposure and recent exposure. We advise that larger studies should take the duration of exposure into account.

One of the strengths of our study is that the research question was translated into a theoretical design and the corresponding method of data collection and method of data processing was chosen. [14,28-30]. We performed an incidence-density study allowing us to sample events and population moments and to reconstruct the antecedents carefully (exposure and other relevant characteristics) before the occurrence of the event or at sampling as a population moment. In order to achieve this, we made use of data from the PIPO project, where extensive information about the pregnancy of the mothers and information on the offspring was collected repeatedly from birth up until the age of 8 years. This extensive information was collected prior to occurrence of the event in the PIPO prospective data collection project, including the information on exposure to ETS. This limits misclassification for the exposure (recall bias, etc.). The extensive information collected at regular time-points in the PIPO project allowed us to take into account relevant exposures and characteristics over the whole life span of the child (modifiers and confounders) at relevant time-points (prior to the diagnosis of asthma). Lastly, we dealt with missing data by using multiple imputation by chained equations for imputation. Performing the modelling on complete cases only would have led to biased results [16].

5.6 Conclusions

In conclusion, we found a strong association between the occurrence of asthma and recent exposure to ETS. This association seems not to be modified by perinatal exposure to ETS. We advise further studies to assess the relationship between asthma and exposure to ETS in more detail (e.g., dose and duration of exposure) with larger sample sizes and with a similar design (theoretical design, design of data collection and design of data processing carefully considering temporal aspects between exposure(s) and outcome).

5.7 Acknowledgments

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Chapter 6: General discussion

With this PhD-project we wanted to add to the discussion on theoretical design in epidemiologic etiologic research by gaining insight into the reporting of theoretical design in literature, the use of theoretical design by researchers and knowledgeability of the concept of theoretical design among researchers. Our specific aims were:

1. To critically appraise the reporting of theoretical design and key elements of theoretical design in epidemiologic etiologic research on the relationship between occurrence of asthma and early life antibiotic use.
2. To appraise perceived knowledgeability of theoretical design among authors publishing research on the relationship between occurrence of asthma and early life antibiotic use.
3. To apply the concepts (discussed and presented in the work for specific aims 1 and 2) in a study on the relationship between occurrence of asthma and use of systemic antibiotics during the first year of life.
4. To apply the concepts (discussed and presented in the work for specific aims 1 and 2) in a study on the relationship between occurrence of asthma and recent exposure to environmental tobacco smoke (one year prior to diagnosis) in children.

Before we elaborated on our research, we presented the theoretical framework for this thesis. As our interest was in epidemiologic observational etiologic research and theoretical design, we focused on event-type health outcomes with 'current occurrence of the outcome as a function of past exposure' being the underlying theoretical design. In this thesis we critically appraise articles regarding the reporting of key elements of theoretical design and the perceived knowledgeability of the concept of theoretical design among authors. Additionally, we also presented a method for sampling incidence density from a study base within an observation period allowing for estimating instantaneous incidence density, applying the presented concepts.

The reporting of theoretical design and key elements of theoretical design was critically appraised in recent studies on the relationship between occurrence of asthma and early life antibiotic use. In the reviewed literature, theoretical design was not reported at all and even key elements of theoretical design were hardly reported (chapter 2). The key elements considered were: measure of occurrence, case (event or state) definition, conceptualization (and operationalization) of the exposure, temporal relation between outcome and exposure, confounders and effect modifiers taken into account and the domain of the study. Comparing the (reviewed) articles published before and after the publication of STROBE,[1] no differences in reporting of these key

elements were observed. The corresponding authors of a selection of the reviewed articles (in chapter 2) were contacted and asked to fill in an informal questionnaire. Questions were asked about the research question of the study, the theoretical design of the study and the selection of confounders and effect modifiers (use of causal theory, confounders adjusted for and effect modifiers taken into account). The aim was to gain insight into the use of theoretical design at conceptualization of a study and in all phases before the first submission of the manuscript (i.e. before peer-review) (chapter 3). The authors were also asked to indicate whether an epidemiologist was involved in the study and whether changes (and which ones) were made to the manuscript as a consequence of the reviewers' comments. While some authors perceived themselves to be extremely knowledgeable with the concept of theoretical design, others indicated to be less or not at all knowledgeable with this concept. However, only one author formulated the theoretical design of their study. Noteworthy was that when occurrence functions were presented to the authors, almost all selected 'current occurrence as a function of past exposure'. The reporting of theoretical design in the articles was apparently not influenced by the reviewers' comments and in almost all studies an epidemiologist was involved.

Two exposure-outcome relationships were investigated, applying the presented concepts (theoretical design and incidence-density study). In the first study (chapter 4) it was observed that children exposed to four or more courses of systemic antibiotics during the first year of life have almost twice the incidence density for asthma occurrence, although borderline significant. This association was much stronger (and significant at the α-level of 0.05) in children reporting lower respiratory tract infections (LRTIs) in the first year of life. In the second study (chapter 5), an association was observed between occurrence of a first doctor's diagnosis of asthma and recent exposure to ETS (one year prior to diagnosis). There was no indication of effect modification by perinatal exposure to ETS. In both studies the research question and theoretical design were explicitly reported and matched to the design of data collection and design of data processing. The explicit use and reporting of the research question and the theoretical design was perceived to be clear by the reviewers. Regarding the conduct of an incidence-density study, matching the theoretical design for both studies, no major concerns were raised by all reviewers. Several reviewers did ask to clarify some of the used terminology, such as 'population moments'. They questioned whether the readers of the journals will understand this terminology and asked to elaborate more on this term.

6.1 Appraisal of reporting of theoretical design and key elements of theoretical design in epidemiologic etiologic research

Object (theoretical) design is not a new concept but was already introduced in textbooks in the 80's.[2] It is an important aspect of study design and is essential for the design of data collection, the design of data processing and the interpretation of study results. Nevertheless, in none of the reviewed articles, a theoretical design was reported (chapter 2). Underreporting of theoretical design could have several explanations. Researchers might not be knowledgeable with the concept of theoretical design. This is plausible, considering the different ways in which study design is interpreted as discussed in the introduction of this thesis. Depending on the training of researchers, there is without doubt some diversity in how researchers apply (even) basic concepts in their studies. Assuming that researchers are knowledgeable with the concept of theoretical design and that the theoretical design was conceptualized when designing the study, researchers might not report the theoretical design in the resulting article. Explicit reporting of the theoretical design might have been considered as irrelevant because guidelines for example do not stress the importance of reporting the theoretical design. The underreporting might also be related to the peer-reviewing process. Manuscripts are adapted taking into account reviewers' comments and if reviewers regard the explicit reporting of theoretical design as irrelevant, this might convince researcher to not report the theoretical design. From the answers of the authors (chapter 3) it was clear however, that the peer-reviewing process did not influence the explicit reporting of theoretical design.

Theoretical design encompasses key elements that are essential for the conduct of a study and that should be reported in an article. These key elements (cf. supra), are basic concepts in epidemiology that should be well known among epidemiologists or researchers conducting epidemiological research. Readers (whether or not researchers) should also be able to interpret a published study and without the reporting of these key elements, this would be hampered. Therefore, it is to be expected that these key elements are explicitly reported in published articles. This will increase the transparency of a study. However, it seems that key elements of theoretical design are reported poorly or not reported at all, even though these are basic concepts in epidemiology. In the reviewed articles we formulated a theoretical design for a selection of articles (chapter 2). However, formulating a theoretical design was not straightforward for articles in which the reporting of the key elements of theoretical design was incomplete or inappropriate. We could not observe any major differences in reporting before and after the publication of the STROBE statement.

Even though these key elements of theoretical design are basic concepts in epidemiology, it seems that there are still some issues in the appropriate reporting of these concepts. The inappropriate or incomplete reporting of these key elements can have several explanations. The three most obvious explanations could be: (1) the lack of proper reporting guidelines, (2) the peer-review process influencing the reporting in the manuscript and (3) the authors' knowledge of these basic concepts.

Regarding the lack of proper reporting guidelines, existing guidelines do stress the importance of proper reporting, but they do not specify what key elements are and what the definition is for these key elements.[1,3] Existing reporting guidelines such as STROBE, are apparently not enough to ensure explicit and proper reporting of theoretical design and key elements of theoretical design. In order to achieve this, reporting guidelines should be more explicit in defining what these key elements are. This will at least provide authors with a guideline on what to report rather than allowing authors the choice in what key elements they report. If reporting guidelines explicitly define what key elements should be reported, this will inevitably increase the quality of reporting.

After surveying the authors of a selection of the articles reviewed in chapter 2, it seemed that the reporting of theoretical design and it's key elements were not influenced by the peer review process (reviewers' comments). Authors generally agreed with reviewers' comments and the changes made to the manuscript as a consequence of these comments were (according to the authors) not related to the reporting of theoretical design. This implies that the incomplete or inappropriate reporting was not noticed by the reviewers. Also, this could be related to the lack of more specific reporting guidelines, but also to the knowledge about these basic concepts among the authors. Concerning the peer review process, the explicit reporting of the theoretical design in our two etiologic studies in this PhD-project (chapter 4 and 5) was not perceived as redundant by the reviewers. The reviewers did not suggest to elaborate on this concept or to make changes to the theoretical design. They rather perceived the reporting to be clear, informing them about what the study is about. One of the reviewers of our critical appraisal on the reporting of theoretical design (chapter 2) explicitly states: *"There is a very important message in this manuscript about the importance of the proper report of the key elements of every study and how these could affect the interpretability of the findings on a possible association between an exposure and an outcome."* As the theoretical design encompasses key elements for conducting and interpreting a study, reporting this early in the methods section probably increases the transparency of the study for other researchers or readers. It facilitates the reviewers to evaluate whether the design of

data collection and the design of data processing matches with the theoretical design of the study and the aim and whether the study results were interpreted correctly. The increased transparency of a study, when theoretical design is explicitly reported, can therefore be considered of added value.

Regarding the possible lack of knowledge among the authors about these basic concepts, this cannot be deduced from what was reported in the articles. Authors might have conceptualized and defined key elements of theoretical design before or during the conduct of their study without reporting them explicitly in the resulting article. After surveying the authors (which will be discussed later), it seemed that not all authors were knowledgeable with the concept of theoretical design. The vast majority of the authors did however perceive themselves to be be knowledgeable with the concepts of confounding and effect modification (which are as well key elements of theoretical design). The authors reported confounders and effect modifiers taken into account during the study. However, which aspect of causal theory they used to select the confounders and effect modifiers was not reported in the vast majority of the articles. Some authors referred to previous publication, but this is not an aspect of causal theory. Aspects of causal theory are for example directed acyclic graphs (DAGs) and the sufficient-component cause model.[4] Confounders and effect modifiers seem to be known concepts among researchers. This is plausible, since confounding and effect modification are considered as essential to take into account when conducting a study (for the validity). It seems obvious therefore, that the vast majority of researchers must be knowledgeable with these concepts in order to conduct the analyses for their study. This is not the case for theoretical design and causal theory, even though these are essential elements in the designing of a study. However, even if authors were not knowledgeable with these concepts or with key elements of theoretical design, an epidemiologist should be involved in epidemiological studies to ensure proper conduct and reporting. For all articles reviewed, an epidemiologist was involved according to the authors. Nevertheless, this does not seem sufficient for adequate reporting of all key elements of theoretical design. The epidemiologist involved in a study might not be one of the authors of the article. If this is the case and if the epidemiologist included only contributed minimally to the article, the lead researchers (authors) of the study must ensure that all important (epidemiological) elements are properly reported. Either way, researchers must ensure proper reporting of all essential elements of a study, which include key elements of theoretical design. For this, the training of researchers should be adequate and should also address theoretical design and key elements of theoretical design.

6.2 Perceived knowledgeability of theoretical design, causal theory, confounding and effect modification among authors

After surveying the authors, we observed that there was diversity in how authors perceived themselves to be knowledgeable with the concepts of theoretical design and causal theory (from not at all knowledgeable to extremely knowledgeable with these concepts). The authors were asked to formulate a theoretical design for the study and to indicate which aspect of causal theory they used for the selection of confounders and/or effect modifiers. Whereas the majority of the authors indicated to be to some extent knowledgeable with the concept of theoretical design, only one author could formulate a theoretical design for their study. For the concepts of confounding and effect modification, the vast majority of the authors indicated to be knowledgeable with these concepts. From what was reported in the articles (chapter 2) and from the answers in the survey (chapter 3) it seems that these two concepts are well known among the authors. The definition of confounding and effect modification in textbooks is more uniform compared to other concepts in epidemiology. The concepts of confounding and effect modification are also covered in introductory courses in epidemiology. This is not the case for the concepts of theoretical design and causal theory, which could also be deduced from the diversity in how authors perceived themselves to be knowledgeable with these concepts.

Apart from what was deduced from the answers of the authors, also the reviewers of the manuscript (chapter 3) commented that the terminology might not be easily understood by readers. More specifically, one of the reviewers refers to the terms 'occurrence function' and 'theoretical design'. At the same time, this reviewer acknowledged that our manuscript discussed an important aspect regarding conduct of research and reporting: *"The commentary discusses a very important aspect of the conduct and reporting of researches. With further explanation on some terms used, this is a good commentary to publish once the relevant concerns are addressed (I acknowledge that some of my concerns may not be relevant to you)."*

The diversity in how authors perceive themselves to be knowledgeable with the concepts of theoretical design and causal theory can be explained by the training of the researchers conducting epidemiological (observational) research. As we discussed in the introduction of this thesis, textbooks on epidemiological concepts and methods may differ in how they define (even basic) concepts in epidemiology. As there is no single 'bible' for basic epidemiology and training programs are free to choose which textbooks they use, it is not surprising that there is diversity among the authors in how

knowledgeable they are with the concepts presented in this PhD-thesis. Training programs might also differ in their focus within the field of epidemiology. Where one training program might focus on the practical aspects of conducting epidemiological studies (how to collect data and how to process the data) within a certain field (e.g. clinical epidemiology, environmental epidemiology), other training programs might also focus on the theoretical concepts in epidemiology. Apart from programs meant to specifically train researchers in epidemiological methods, other training programs for health professionals (e.g. medicine) include some courses on research methodology (as pointed out in chapter 3). However, these courses might not be in-depth enough in order to properly prepare health care professionals or other scientists to conduct high-quality epidemiologic research. Also, besides having a relevant background (usually a master's degree in the (bio)medical field) no rules are set for who can conduct epidemiological studies. This is relevant to reflect on, especially regarding methodological training essential for properly designing, conducting and reporting epidemiological studies.

6.3 Application of the concepts presented in this PhD-project in epidemiologic etiologic research

Important basic concepts (theoretical design, incidence-density study) in epidemiologic etiologic research were presented in the introduction as the framework for this PhD-project. From reviewing articles and surveying the authors it was clear that the authors did not conceptualize a theoretical design and that the peer review process was not influencing the reporting of a theoretical design. The vast majority of these authors was not able to formulate a theoretical design. Therefore, we checked off whether the authors were able to understand and conceptualize theoretical design (chapter 3) and whether readers (i.e. the reviewers) of the articles resulting from our two etiologic studies (chapter 4 and 5) would appreciate the importance of the concepts presented.

When presenting authors with occurrence functions (part of the theoretical design) from which they could choose from (by selecting which occurrence function best suits their study), almost all authors selected 'current occurrence as a function of past exposure'. This implies that the interest is in assessing a relationship between a certain outcome and antecedental exposures of that outcome. When authors were presented with occurrence functions, it indeed seemed obvious to them to select 'current occurrence as a function of past exposure' for their study which was of etiologic nature. This was however not reflected in how the authors conducted the study. This observation can be explained by the RCT-dogma. Researchers aim to (and are very

eager to) conduct a cohort study because they consider this to be the next best option when conducting a randomized controlled trial (RCT) is unfeasible or unethical. The occurrence function for this 'cohort study' would then be 'future occurrence as a function of current exposure'. This is not the occurrence function of an etiologic study, because the interest there is in explaining current occurrence by first identifying the events or states followed by reconstructing antecedental exposure(s). The authors clearly understood this concept of theoretical design for epidemiologic etiologic research, given that they all selected 'current occurrence of the outcome as a function of past/current exposure' when presented with several occurrence functions. Also, from the reviewers' comments regarding our work in chapter 4 and 5, the explicit reporting of a theoretical design for both (etiological) studies was perceived to be clear.

After conceptualization of a theoretical design, the design of data collection and the design of data processing should be conceptualized as well. These should match with the theoretical design of the study. If no theoretical design was conceptualized, the design of data collection and design of data processing might not match the underlying theoretical design of a study (in this case the theoretical design that can be deduced from the study will be depending on the design of data collection and not the other way around, which is incorrect). In chapter 4 and 5 of this thesis, the design of data collection and the design of data processing matched the theoretical design. In both chapters we decided to consider asthma as an event-type of illness for different reasons. An incidence-density study was conducted in both studies, since both were of etiologic nature. The reviewers perceived this to be clear and commented that the design of data collection and design of data processing matched with the aim, research question and the theoretical design. The reviewers did ask to elaborate more on the terms 'population time' and 'population moments' out of concern that readers might not fully understand these terms (even though an explanation and a reference were provided). One of the reviewers indicated that he/she had to go back to the textbooks to understand the terms:

> *"POPULATION MOMENT: I am not familiar with this term. It appears to represent individuals that are controls. This should be better described so that the reader/reviewer has better understanding of the term"* (reviewer 1)
>
> *"As a comment from readership perspective, I must say that use of statistical terminology such as incidence density and population moments sent me back to textbooks to try and understand the intent for that usage. After much perusal I am skeptical that the average reader of the Journal will appreciate the*

terminology or the rationale for its use. At a minimum clear definitions should be provided." (reviewer 2)

"I strongly suggest that the authors include a short explanation of the concept of "population moments" either in the methods section or the online supplement material. This will greatly benefit target readers of this journal, which may not have a statistical background, or may not be familiar with quasi-experimental designs." (reviewer 3)

The reviewers stated that they doubt whether the readers of the journal will understand the terminology used. The concept of population moments was therefore explained in the manuscripts, emphasizing that population moments represent the population time at risk for the occurrence of the event (the study base) and not the individuals contributing to that population time. It seems that the reviewers are not quite familiar with this concept as well. One of the reviewers interpreted the study as a quasi-experimental design. A quasi-experiment is an experiment where the researcher is not able to have control over the allocation (random assignment) of an intervention (i.e. a so called 'cohort study').[5] The study is then conducted as if it were to be an experiment. This is definitely not what an incidence-density study is about.

Even though the reviewers raised some concerns about whether the terminology would be understood by the readers of the journals, it seems that the presented concepts in this PhD-project are perceived as clear and are understood by authors and reviewers of epidemiologic etiologic studies once presented and explained to them. However, the concepts presented are not generally applied in studies. By not explicitly formulating a theoretical design for their study, there is the chance that the design of data collection and design of data processing rather matches a study where the theoretical design would be 'future occurrence as a function of current exposure' (studying current exposure as a predictor, prognostic indicator for later occurrence, i.e. a prognostic study), rather than an etiologic study (studying the occurrence as a function of past exposure). This might also have some impact on the interpretation of the study results. Based on the work in this PhD-project we cannot draw conclusions about the size of the impact on study results. For this, it would be of interest to conduct simulation studies, simulating different scenario's and assessing whether there is an effect on the resulting estimates. Regardless of whether there would be a major impact on the study results, it is still important to understand, use and report important aspects of study design when conducting research. This for (at a minimum) the sake of transparency of research.

Another important aspect to consider in the discussion about the application (conceptualization and explicit reporting) of theoretical design is the training of

researchers (cf. supra). It is important to critically review training programs for researchers that will be conducting epidemiological research. These programs should include the teaching of basic concepts of epidemiology such as the theoretical design of a study within the framework for an epidemiologic etiologic study. Regular evaluation of existing training programs should be a matter of course, which is for example the case in the Netherlands. Training programs in epidemiology are regularly evaluated by the Netherlands Society for Epidemiology (VvE). In order for these evaluations to be successful however, there should also be more rigid definitions of basic epidemiological concepts, which (as discussed earlier) is partly the responsibility of academics.

6.4 The importance of theoretical design in epidemiologic etiologic research

Based on what was observed within this PhD-project, we can conclude that theoretical design is underreported and that researchers are not knowledgeable with this concept. Theoretical design can be considered as a basic concept in epidemiology and should be conceptualized and delineated before the conduct of a study and must be reported explicitly in the resulting manuscript. The design of data collection and design of data processing must match the theoretical design. The theoretical design is also the backbone for the interpretation of study results. Failure to conceptualize a theoretical design for a study can lead to a mismatch between the (not explicitly conceptualized, but underlying) theoretical design and the design of data collection and data processing.

As theoretical design is not reported at all in the articles reviewed in this PhD-project, we propose that a shift in the approach when designing, conducting and reporting a study is necessary in epidemiologic etiologic research. Epidemiologic etiologic research is often conducted by researchers with a background in medicine, which is a field focused on health care. Physicians are trained to care for (and treat) patients by first taking the patient's personal medical history (measuring what has happened before the patient became 'ill'), i.e. the anamnesis of the patient. Subsequently, the physician tries to assign a diagnosis to the patient. In medicine therefore, the emphasis is not on starting from a theoretical framework. The approach is different once we are in the field of medical science. In this field it does not concern individual patients, but populations instead. The results from medical research are used to inform physicians and to aid them in the process of diagnosis and treatment of individual patients. The studies conducted with populations need to be designed properly before conducting

them and this involves the conceptualization of the theoretical framework (i.e. theoretical design). The results of studies need to be interpreted taking the theoretical design into account. In other fields, researchers are more familiar with starting from a theoretical framework before conducting a study. Statisticians for example take into account the statistical assumptions about the data when using a certain method. Social scientists consider the research paradigm before conducting a study. A research paradigm or statistical assumption is not the same as a theoretical design, but at least attention is given to what the used methods mean for the results before interpreting the results.

In the field of epidemiology, there is still a lack of consensus on what epidemiologic etiologic research is and how an epidemiologic etiologic study should be conceptualized and conducted. Considering the theoretical design will clarify this among researchers. Conceptualizing a theoretical design will facilitate the design of data collection and the design of data processing. Not conceptualizing a theoretical design will possibly result in conducting a study inspired by the 'RCT-dogma', i.e. try to mimic a 'cohort study' as much as possible. Explicit reporting of the theoretical design might also lead to an increase in transparency of medical research and an increase in the quality of reporting. Eventually, this might result in an increase of the quality of medical research.

The concepts discussed in this PhD-project are not advanced, but rather basic concepts that researchers conducting epidemiologic etiologic research should have basic understanding of. From what was observed in this project, we get the impression this understanding is rather limited. How to improve the definition of basic concepts in epidemiology and how to properly train researchers is a discussion that needs to be held among epidemiologists in academia. This is part of the responsibility of academics. The way epidemiologic research is conducted currently, the definition of basic concepts and the direction towards we are heading in epidemiology are matters that should be looked at with a critical eye. This, with the aim of improving the quality of epidemiologic research and eventually reducing research waste. In this thesis we attempted to take our responsibility as academics by aiming to raise the matter of the importance of theoretical design and to add to the discussion. We hope very much that we have succeeded in this to some extent.

6.5 References

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7. Appendices chapter 2

7.1 Appendix 1: List of all articles (n = 63) included in the critical appraisal.

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7.2 Appendix 2: table with all the extracted information from the reviewed articles.

List of abbreviations used in the table

AB	Antibiotics/antibiotic
BMI	Body mass index
COPSAC	The Copenhagen Prospective Studies on Asthma in Childhood
DNBC	Danish National Birth Cohort
ECRHS	European Community Respiratory Health Survey
ETS	Environmental tobacco smoke
GP	General practitioner
HR	Hazard ratio
ICD	International classification of disease
ICS	Inhaled corticosteroids
ISAAC	International Study on Asthma and Allergies in Childhood
KPNC	Kaiser Permanente Northern California
LMP	Last menstrual period
LRTI	Lower respiratory tract infection
MD	Medically diagnosed
MMR	Measles mumps rubella
NICU	Neonatal intensive care unit
NSAIDs	Non-steroidal anti-inflammatory drugs
OR	Odds ratio
PACMAN	The Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory Effects

RTI	Respiratory tract infection
SES	Socio economic status
SGA	Small for gestational age
SPT	Skin prick test
URTI	Upper respiratory tract infection
UTI	Urinary tract infection

Part 1 of the table with all extracted information from the reviewed articles (Italic font indicates text quoted from an article; /: not reported.)

Nr.	Journal	Year of publication	Title of the article	Aim of the study	Research question or hypothesis of the study
1	BMC pregnancy and childbirth	2018	The effect of breastfeeding on the risk of asthma in high-risk children: a case-control study in Shanghai, China	*"…to investigate the association between AB use in pregnancy and the risk of childhood asthma, and the possible role of breast feeding in modulating the risks…"*	/
2	Allergy, Asthma & Immunology Research	2018	Effects of antibiotics on the development of asthma and other allergic diseases in children and adolescents	*"…determine if the duration of exposure to AB in children and adolescents is associated with the later development of allergic disease."*	/
3	Journal of asthma	2018	The current prevalence of asthma, allergic rhinitis, and eczema related symptoms in school-aged children in Costa Rica	*"…to study the current prevalence 12 years later in an inner-city group of children between 6 and 13 years old."*	*"Our hypothesis is that similar to other Latin American countries the prevalence of both asthma and allergic rhinitis in Costa Rica has increased."*
4	European Respiratory Journal	2018	Prenatal antibiotic exposure and childhood asthma: a population-based study	*"…examining the association of maternal AB use and childhood asthma…"*	/
5	Scientific Reports	2018	Effect of antibiotic use for acute bronchiolitis on new-onset asthma in children	*"…to assess the relationship of early life AB use for bronchiolitis with new-onset asthma in children."*	/
6	JAMA Pediatrics	2018	Association between use of acid-suppressive medications and antibiotics during infancy and allergic diseases in early childhood	*"…to evaluate the hypothesis that exposure to either acid-suppressive medications or AB during infancy is associated with an increased risk of childhood allergic disease…"*	*"…exposure to either acid-suppressive medications or AB during infancy is associated with an increased risk of childhood allergic disease…"*
7	Pediatric Allergy and Immunology	2018	Prenatal and early-life antibiotic use and risk of childhood asthma: a retrospective cohort study	*"…evaluate the association between AB exposure in early life and asthma development in childhood considering the types of AB…"*	/
8	Acta Pediatrica	2018	Antibiotics in the first week of life were associated with atopic asthma at 12 years of age	*"…examine the prevalence and risk factors for asthma at 12 years and to examine associations with atopic asthma and non-atopic asthma…to analyze whether the long-term effects of AB during the first week of life could be seen at age 12."*	/
9	Annals of Allergy, Asthma, & Immunology	2017	Influence of antibiotic use in early childhood on asthma and allergic diseases at age 5	*"…to elucidate the relation between postnatal AB exposures within the first two years of life and allergic disease in children at 5 years of age…"*	/
10	Pediatric Allergy & Immunology	2017	Early life antibiotic use and the risk of asthma and asthma exacerbations in children	*"…evaluate the effect of early life AB use on the risk of asthma onset later in life in the general population….in the pediatric population with asthma, the association between early life AB use and the risk of asthma exacerbations later in life was studied."*	/
11	International Journal of Gynecology and Obstetrics	2017	Evaluation of the associations between childhood asthma and prenatal and perinatal factors	*"…to identify prenatal and perinatal risk factors of childhood asthma."*	/
12	The Journal of Allergy and Clinical Immunology: In Practice	2017	Antibiotic use in early life, rural residence, and allergic diseases in Argentinean children	*"…examine the relation between antibiotics use in early life and current asthma among children aged 6 to 7 years…"*	*"…we hypothesized that antibiotic use would affect the risk of atopic diseases (current wheeze or allergic rhino-conjunctivitis) among children living in urban areas in early life, but not among those living in rural areas in early life…."*

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13	Allergy & Asthma Proceedings	2016	Hygiene factors associated with childhood food allergy and asthma	*"…to assess whether hygiene factors are associated with a food allergy and/or asthma diagnosis. We investigated key hygiene factors, including AB use, and their association with food allergy and asthma…"*	/
14	Clinical & Experimental Allergy	2016	Antibiotic use during pregnancy and asthma in preschool children: the influence of confounding	*"…to assess the association between AB use in pregnancy and the development of asthma in preschool children,… evaluate the influence of confounding, …we evaluated the influence of potential time trends in exposure frequencies in the case-sibling analysis."*	*"We hypothesized that if an effect would be present, that this would be strongest in the third trimester, as antibiotic use during in late pregnancy likely has the strongest influence on the infants' intestinal flora."*
15	Plos One	2016	Relative importance and additive effects of maternal and infant risk factors on childhood asthma	*"…determine the relative impact and cumulative effect of in utero, perinatal and postnatal exposures that could be measured during pregnancy and infancy on the risk of developing early childhood asthma: maternal AB use/urinary tract infection (UTI), mode of delivery, infant AB use, and having older siblings. We also assessed the dose-dependent relationship of maternal AB use/UTI, infant AB use, and number of older siblings on the risk of developing early childhood asthma"*	/
16	Nature Communications	2016	Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children	*"…to investigate the short- and long-term effects of AB on preschool children's intestinal microbiome and health."*	/
17	Plos One	2015	Periconceptional and gestational exposure to antibiotics and childhood asthma	*"…to assess the associations between maternal exposure to different types of AB before and during pregnancy and childhood asthma..."*	/
19	Allergy, Asthma & Immunology Research	2015	Association between antibiotic exposure, bronchiolitis, and TLR4 (rs1927911) polymorphisms in childhood asthma	*"…investigate the risk factors involved in the development of asthma during early life and their interactions…., we investigated whether AB exposure in the first year of life and a history of physician diagnosed bronchiolitis in the first 2 years of life were associated with an increased risk of childhood asthma. …, we evaluated whether a polymorphism in TLR4 (rs1927911) modulates the impact of these environmental factors…"*	/
20	Annals of Allergy, Asthma, & Immunology	2015	The relationship between prenatal antibiotic use and asthma in at-risk children	*"…we investigated the effects of prenatal AB use with the subsequent development of asthma by year 3 and wheezing in the third year…"*	/
21	BMJ	2014	Antibiotics in fetal and early life and subsequent childhood asthma: nationwide population based study with sibling analysis.	*"We examined the association between exposure to AB in fetal life or childhood and subsequent development of asthma…"*	/
22	Epidemiology and Health	2014	Effects of antibiotics consumption on children 2-8 years of age developing asthma	*"…the primary objective was to investigate the association between AB exposure and the risk of developing childhood asthma."*	/
23	Clinical and Experimental Allergy	2014	Prenatal and post-natal exposure to antibiotics and risk of asthma in childhood	*"To assess the association between both prenatal and postnatal exposure to AB and to explore the role of different classes of AB with the risk of asthma in childhood.."*	/

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24	The Lancet Respiratory Medicine	2014	Assessing the association of early life antibiotic prescription with asthma exacerbations, impaired antiviral immunity, and genetic variants in 17q21: a population-based birth cohort study	*"... further clarify the complex relation between early life antibiotic use and asthma."..."... explored the association between AB prescription and the development of wheezing, severe asthma exacerbations and atopy during childhood, investigated whether there are differences in antiviral and antibacterial immune responses between children who received AB in infancy compared with those who did not; and explored the association between AB prescription and polymorphisms in chromosomal locus 17q21."*	*"We postulated that antibiotic prescription in early life is a proxy marker of impaired antiviral immunity and increased susceptibility to recurrent or more severe viral infections, which might, in part, be genetically determined, and that this susceptibility to viral infection (rather than antibiotic prescription or a virus per se) increases the risk for persistence of asthma symptoms and severe asthma exacerbations."*
25	The Lancet Respiratory Medicine	2014	Maternal propensity for infections and risk of childhood asthma: a registry-based cohort study	*"...analysing associations between maternal use of AB and the occurrence of asthma in the offspring in a period from 80 weeks before pregnancy, during pregnancy, and up to 80 weeks post-partum..."*	*"... if maternal antibiotic use was causally associated with asthma in children, the recorded association would be strongest for antibiotic use during, or shortly before, the pregnancy period...."*
26	Pediatric Allergy & Immunology	2013	Antibiotic exposure in the first two years of life and development of asthma and other allergic diseases by 7.5 yr: a dose-dependent relationship	*"...we evaluated the association between timing and number of courses of infant AB exposure and the development of asthma and allergic diseases...".*	/
27	International Journal of Epidemiology	2013	Acetaminophen and/or antibiotics use in early life and the development of childhood allergic diseases	*"...we investigated the temporal relationship between the use of acetaminophen and/or AB use during the first year of life and the development of atopic dermatitis, allergic rhinitis and asthma, respectively, in later childhood (2-6 year olds).*	/
28	Advances in Experimental Medicine and Biology	2013	Exposure to paracetamol and antibiotics in early life and elevated risk of asthma in childhood	*"...to examine the association between AB and paracetamol intake in the first year of life and the development of asthmatic symptoms in childhood."*	/
29	Pediatric Allergy and Immunology	2013	Early fish introduction and neonatal antibiotics affect the risk of asthma into school age	*"...explore the impact of early life events on the risk of asthma at 8 years, with special reference to the early introduction of fish and treatment with AB neonatally....only broad-spectrum AB given during the first week of life were considered."*	/
30	International Journal of Occupational Medicine and Environmental Health	2012	The relationship between antibiotic therapy in early childhood and the symptoms of allergy in children aged 6-8 years – the questionnaire study results	*"...to assess the relationship between the use of AB in early childhood and the prevalence of allergies, asthma, and their associated symptoms in the urban early-primary-school age group in Poland."*	/
31	The Journal of Pediatrics	2013	Use of antibiotics during pregnancy increases the risk of asthma in early childhood	*"...We therefore tested the hypothesis that maternal use of AB during pregnancy was associated with increased risk of asthma and eczema during early childhood ... we also examined the associations in the subset of mothers using AB for non-respiratory infections."*	*"...the hypothesis that maternal use of antibiotics during pregnancy was associated with increased risk of asthma and eczema during early childhood..."*
32	International Archives of Allergy and Immunology	2012	Prevalence of atopy and allergic diseases in Korean children: associations with a farming environment and rural lifestyle	*"...to compare the prevalence of allergic diseases and atopic sensitization among Korean schoolchildren in an urban city, a rural town and a rural village. We investigated the association of putative risk and protective factors with allergic diseases and atopic sensitization in these children."*	*"We hypothesized that the prevalence of allergic diseases and atopic sensitization would be lower among rural children in Korea due to differences in environment and lifestyle."*
33	Clinical and Experimental Allergy	2011	Antibiotics and asthma medication in a large register-based cohort study – confounding, cause and effect	*"...to estimate the association between prescribed AB and asthma medication..."* *"We also intended to address the issues of reverse causation and confounding by indication..."*	/

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34	Asian Pacific Journal of Allergy and Immunology	2011	Prevalence and risk factors for early presentation of asthma among preschool children in Taiwan	*"...to understand current conditions and prevalence of allergic diseases among preschool children and to identify putative causative factors for these allergic symptoms..."*	/
35	Journal of Physiology and Pharmacology	2011	Wheezing and asthma may be enhanced by broad spectrum antibiotics used in early childhood. Concept and results of a pharmacoepidemiology study.	*"...to investigate the relationship between different classes of AB treatment in early childhood and the medical diagnosis of asthma or wheezing reported by mothers..."*	*"The primary hypothesis was that broad spectrum antibiotics compared to narrow spectrum may increase the risk for asthma in early childhood."*
36	Allergy, Asthma, and Immunology Research	2011	Changes in the prevalence of childhood asthma in Seoul from 1995 to 2008 and its risk factors	*"We determined the prevalence of wheezing and asthma among elementary school children in 5 areas of Seoul, and compare it to the results reported by the 1995, 2000 and 2005 ISAAC surveys in Korea. ..., we compared the prevalence among the aforementioned 5 areas and investigated the risk factors for wheezing and asthma."*	/
37	American Journal of Epidemiology	2010	Antibiotic exposure by 6 months and asthma and allergy at 6 years: findings in a cohort of 1401 US children	*"To reduce risk of protophatic bias, we assessed the association of AB use within the first 6 months of life with asthma and allergy at 6 years of age. We considered whether the association differed according to parental history of asthma."*	*"We hypothesize that early antibiotic use is associated with increased risk of childhood asthma."*
38	Clinical and Experimental Allergy	2010	Antibiotics use in early life and development of allergic diseases: respiratory infection as the explanation	*"...to investigate the relationship between AB-use during the first year of life and the development of allergic disease..."*	/
39	Clinical and Experimental Allergy	2010	Relation of antibiotic use to childhood asthma: confounding by indication?	*"...we examined the association between oral AB use in the first 9 months of life and the development of physician diagnosed asthma, physician diagnosed eczema and allergic allergen sensitization by 5 years of age..."*	/
40	Allergy, Asthma, and Immunology Research	2010	Prevalence of childhood asthma in Korea: international study of Asthma and Allergies in childhood	*"...the prevalence of childhood asthma and its risk factors in Korea are described and compared with those from other countries."*	/
41	Paediatric and Perinatal Epidemiology	2010	Neonatal sepsis, antibiotic therapy and later risk of asthma and allergy	*"... to investigate the selective contribution of neonatal sepsis and AB therapy on the risk of atopic dermatitis, hay fever and asthma in children and young adults."*	*"We hypothesised that neonatal sepsis may be associated with a reduced risk of allergic disease in later life."*
42	Journal of Allergy and Clinical Immunology	2009	Antibiotic use in infancy and symptoms of asthma, rhinoconjunctivitis, and eczema in children 6 and 7 years old: International study of asthma and allergies in childhood phase III	*"...to study the associations between parental-reported AB use in the first year of life and current symptoms of asthma, rhinoconjunctivitis, and eczema in children 6 and 7 years old..."*	*"...the hypothesis that antibiotic use may be linked to subsequent risks of asthma, rhinitis, and eczema,..."*
43	Journal of Pediatrics and Child Health	2009	Cross-sectional survey of risk factors for asthma in 6-7 year-old children in New Zealand: International study of asthma and allergies in childhood phase three	*"...to identify and quantify risk factors for asthma in over 10000 6-7-year-old children..."*	/

Nr.	Journal	Year of publication	Title of the article	Aim of the study	Research question or hypothesis of the study
44	Pediatrics	2009	Antibiotic use in children is associated with increased risk of asthma	*"…explore the association between exposure to AB in the first year of life and the subsequent development of asthma… … to evaluate the association between antibiotics prescribed within the first year of life and the development of asthma…"*	*"The primary hypothesis was to explore the association between antibiotic exposure before 1 year of age and development of childhood asthma."*
45	Clinical and Experimental Allergy	2008	Antibiotic use in the first year of life and risk of atopic disease in early childhood	*"…to investigate the association between AB use in the first year of life and the development of atopic disease at 5 years."*	/
46	American Journal of Epidemiology	2008	Determinants of the incidence of childhood asthma: a two-stage case-control study	*"… identifying the independent effects of 47 potential predictors, measured during pregnancy and after birth, on the incidence of asthma development in children within the first 10 years of life."*	/
47	Clinical and Experimental Allergy	2008	The association of early life exposure to antibiotics and the development of asthma, eczema and atopy in a birth cohort: confounding or causality?	*"…to investigate the relationship between AB and the subsequent development of asthma, eczema and atopy… A secondary aim was to determine whether this association is due to confounding by chest infections in infancy."*	/
48	Clinical and Experimental Allergy	2007	Risk factors for asthma at 3.5 and 7 years of age	*"…to examine risk factors for asthma in children aged 3.5 and 7 years of age, with particular emphasis on environmental exposures in infancy including birth weight, maternal smoking, breast feeding, day care, AB treatment and exposure to inhaled allergens."*	/
49	Chest	2007	Increased risk of childhood asthma from antibiotics use in early life	*"…examine the association between oral AB use in the first year of life and asthma at age 7 years…"*	*"We hypothesize that lesser contact with dogs during infancy results in a lower microbial load and makes infants more vulnerable to the effects of antibiotics, especially if they are BS cephalosporins."*
50	Allergy and Asthma Proceedings	2006	Identification of asthma risk factors in Mexico City in an International Study of Asthma and Allergy in Childhood survey	*"…performed a risk analysis based on the ISAAC survey in Mexico."*	/
51	Journal of Allergy and Clinical Immunology	2005	Allergic disease and sensitization in Steiner school children	*"…to identify possible protective factors for allergy associated with the anthroposophic lifestyle.."*	/
52	Indian Pediatrics	2004	Prevalence and risk factors of asthma and wheeze in school-going children in Lucknow, North India	*"…to assess the prevalence of asthma and wheeze and factors associated with it in children aged 6-7 and 13-14 years.*	/
53	Journal of Epidemiology and Community Health	2004	Infections, medication use, and the prevalence of symptoms of asthma, rhinitis, and eczema in childhood	*"We have therefore investigated the association between infections at age 0-4 years, medication use (AB and paracetamol) early in life, and the subsequent risk of childhood asthma at age 6-7 years."*	/

Nr.	Journal	Year of publication	Title of the article	Aim of the study	Research question or hypothesis of the study
54	Clinical & Experimental Allergy	2004	Antibiotics use in the first year of life and asthma in early childhood	*"We have examined the association between the use of oral AB in the first year of life and asthma at ages 2 and 5 years..."*	/
55	International Journal of Epidemiology	2004	Antibiotic sales and the prevalence of symptoms of asthma, rhinitis and eczema: the International Study of Asthma and Allergies in Childhood (ISAAC)	*"We have therefore conducted an ecological analysis of the relationship between AB-sales and the prevalence of symptoms of asthma, allergic rhinitis and atopic eczema..."*	/
56	Thorax	2004	Early prescriptions of antibiotics and the risk of allergic disease in adults: a cohort study	*"...to unravel the direction of associations between prescription of AB and allergic disease."*	/
57	American Journal of Respiratory and Critical Care Medicine	2002	The importance of prenatal exposures on the development of allergic disease	*"...to investigate the relationship between a variety of exposures that alter microbial load during pregnancy, including diagnosis of infections and prescriptions for AB and the incidence of asthma, eczema and hay fever in the child."*	/
58	American Journal of Respiratory and Critical Care Medicine	2002	Lack of association between antibiotic use in the first year of life and asthma, allergic rhinitis, or eczema at age 5 years.	*"...we examined the association between frequency of use of oral AB in the first year of life and asthma, recurrent wheezing, allergic rhinitis; and eczema at age 5 years among study participants."*	/
59	Journal of Allergy and Clinical Immunology	2002	Early exposure to infections and antibiotics and the incidence of allergic disease: a birth cohort study with the West Midlands General Practice Research Database	*"...we examined whether exposure to infections –either personal infections or siblings' infections- protect against the risk of developing allergic disease and whether early exposure to AB increases the subsequent risk of allergic disease.."*	*"Our primary hypothesis was that exposure to infections and antibiotics during the first year of life would affect the subsequent incidence of allergic disease."*
60	Clinical and Experimental Allergy	2000	Does the use of antibiotics in early childhood increase the risk of asthma and allergic disease?	*..."we assessed the association between the use of AB in early childhood and the subsequent development of asthma and allergy in a population-based sample of 7-8 year old schoolchildren.*	/
61	European Respiratory Journal	1999	Frequency of infections and risk of asthma, atopy and airway hyper responsiveness in children	*"...to relate the number of early childhood episodes of fever and AB treatment to the prevalence of asthma, atopy, baseline pulmonary function and airway hyper responsiveness at school age..."*	/
62	Clinical and Experimental Allergy	1998	Antibiotic use in early childhood and the development of asthma	*"... the association between AB use in infancy and the subsequent risk of developing asthma by age 5-10 years has been investigated in children attending Rudolf Steiner schools."*	*"The primary hypotheses of the study concerned the relationship between antibiotic use, vaccinations and the development of asthma."*
63	Thorax	1998	Early childhood infection and atopic disorder	*"To investigate the putative relationship between childhood infections and atopic disorders further..."*	/

Part 2 of the table with all extracted information from the reviewed articles

Nr.	Do the authors mention "(study) design" in the text (+ location)	Case (event or state) definition for asthma	Scientific T_0 (explicitly/implicitly mentioned + temporal aspects)	Measure of disease occurrence (+ abstract temporal aspects)	Exposure conceptualization (+ abstract temporal aspect)	Operationalization of the exposure	Measurement method exposure	Measure of association
1	Yes (methods & discussion)	Childhood asthma	At 6 months of age (referred to as baseline, explicitly)	/ (fuuture incidence)	Maternal AB use in pregnancy (current exposure)	Dichotomous (Yes/No)	Face-to-face interview	OR
2	Yes (discussion)	Asthma diagnosis	Onset asthma (the day allergic diseases were first diagnosed was set as an index date) or before the last day of 2015 (for controls) (explicitly)	Incidence (current incidence)	Duration of AB exposure 7 years prior to development of allergic disease (past exposure)	Annual average AB prescription days (6 categories)	National Health Insurance database	OR
3	Yes (discussion)	Diagnosis of asthma (wheezing or whistling in the chest in the previous 12 months)	Onset asthma (implicitly)	Prevalence (current prevalence)	AB use in the first 12 months of life (past exposure)	Dichotomous (Yes/No)	ISAAC questionnaires at 6-13 years of age	OR
4	Yes (methods)	Child asthma = asthma after the age of 5: any hospitalization for asthma or at least 2 physician diagnoses of asthma (at least 3 months apart and within 1 year) or at least 2 asthma medication prescriptions within 1 year	Birth child (implicitly)	Incidence rate (future incidence)	Maternal AB use during pregnancy (current exposure)	Dichotomous (Yes/No)	From database (prescriptions)	HR Incidence rate
5	No	New-onset asthma: ICD 9 criteria AND receipt of selective B2-agonist and/or ICS treatment twice within 1 year (age 2-18)	Asthma onset (implicitly)	/ (current incidence)	AB dosage AB prescriptions (at least one AB in the 5 years before the onset of asthma) (past exposure)	Cumulative defined daily dose (3 categories) Dichotomous (Yes/No)	Outpatient prescription database	OR
6	Yes (abstract)	Asthma: after the age of 6 months (ICD)	At 6 months of age (implicitly)	Incidence (future incidence)	AB prescription at any time prior to the age of 6 months (current exposure)	Categorical (cut off 10 days)	Tricare Management Activity Military Health System prescription database	HR
7	Yes (introduction & methods)	Incidence of asthma: up to age 6 (diagnosis ICD & ICS and controllers use) (at 12-35 months and 36-72 months of age)	At 12 months of age (explicitly)	Incidence (future incidence)	Prescription of AB to the mother during pregnancy or the child during the first year of birth (current exposure)	Dichotomous (Yes/No) Type of AB (4 categories) Number of AB prescriptions (3 categories)	Large-scale claim database	HR
8	No	'Current' asthma at age 12 years: Atopic 'current' asthma at age 12 Non-atopic 'current' asthma at age 12	12 years of age/1 year of age (implicitly)	Prevalence (current prevalence/future prevalence)	AB treatment in the first week of life (past exposure/current exposure)	Dichotomous (Yes/No)	Questionnaire at 6 months of age	OR
9	Yes (discussion)	'Current' asthma: (past 12 months) at 5 years of age	At 2 years of age (implicitly)	/ (future prevalence)	History of AB use (antibiotics ever in the first 2 years of life) (current exposure)	Dichotomous (Yes/No) Classes of AB (4 categories)	Questionnaire at 2 years of age	OR

Nr.	Do the authors mention "(study) design" in the text (+ location)	Case (event or state) definition for asthma	Scientific T_0 (explicitly/implicitly mentioned + temporal aspects)	Measure of disease occurrence (+ abstract temporal aspects)	Exposure conceptualization (+ abstract temporal aspect)	Operationalization of the exposure	Measurement method exposure	Measure of association
10	Yes (methods & discussion)	Physician diagnosed asthma and asthma exacerbations: Generation R: ever asthma? Confirmed by doctor? (age 9-10) Seaton: ever asthma? Confirmed by doctor? (age 10) Pacman: asthma exacerbations in the past 12 months (age 4-12 years) (emergency department visits, oral corticosteroids) Breathe: asthma exacerbations in the past 6 months (age 3-19 years)	Generation R: at 1 year of age Seaton: at 6 months of age Pacman & Breathe: at 3 years of age (implicitly)	Prevalence rate Cumulative incidence rate (future prevalence)	Antibiotics use (all 4 cohorts) Timing of AB use (Generation R, Pacman and Breathe) Number of prescriptions during first 3 years of life (Pacman and Breathe) Total days of exposure to AB in the first 3 years of life (Pacman and Breathe) (current exposure)	Dichotomous (exposed vs. never exposed) Timing first AB use (1st, 2nd, 3rd year of life) (Generation R, Pacman & Breathe) Number of prescriptions during first 3 years (Pacman & Breathe) (and total days of exposure)	Generation R: parental questionnaire at 1, 2 and 3 years Seaton: first 6 months parent-reported Pacman & Breathe: pharmacy data from first year of life	OR
11	Yes (discussion)	Diagnosis of asthma (age 7-14 years)	Asthma prevalence diagnosis/birth child (implicitly)	/ (current prevalence/future prevalence)	Not specified in methods In tables: AB during pregnancy (past exposure/current exposure)	Dichotomous (Yes/No)	Maternal data sheets from hospitals where children were delivered	OR
12	Yes (methods & discussion)	'Current asthma': parental report of physician diagnosed asthma and current wheeze (in previous year)	6-7 years of age/at 1 year of age (implicitly)	/ (current prevalence/future prevalence)	Any use of AB in the first year of life (past exposure/current exposure)	Dichotomous (Yes/No)	Spanish ISAAC questionnaire distributed at school for parents of 6-7 year old children	OR
13	Yes (introduction)	Asthma: physician diagnosis of asthma (0-21 years) (current)	Inclusion in study (asthma occurrence, implicitly)	Prevalence (current prevalence)	AB use in the first year of life (past exposure)	Dichotomous (Yes/No)	Questionnaire at the time of inclusion	Prevalence ratio's
14	Yes (introduction, methods, results, discussion & appendix)	Asthma: at least 3 prescriptions for asthma medication within a 12 month period before the 5th birthday	Birth child (implicitly)	/ (current prevalence)	Maternal exposure to AB: at least 1 day supply of systemic AB during pregnancy (past exposure)	Dichotomous (Yes/No) Per trimester of exposure Per subgroup of AB (5 classes)	Prescription database	OR
15	No	Childhood asthma: ascertained between 4,5 and 6 years: ICD9 diagnosis or prescriptions for any short-acting β-agonist, 2 prescriptions for montelukast in a 365-day period, or a single prescription of any other asthma-specific medication	Age 1/age 5 (implicitly)	/ (future prevalence/current prevalence)	AB use during infancy (first 12 months) AB use during pregnancy (current exposure/past exposure)	Number of courses of AB (9 categories, from 0 to more than 8 doses for AB use in infancy and 6 categories for AB use during pregnancy)	Infant AB use: medical claims prescription fill data Mothers: Tenn Care insurance	OR
16	No	Diagnosed asthma: current or developing asthma (age 2-7 years)	Asthma diagnosis/at 2 years of age (implicitly)	/ (current prevalence/future prevalence)	Overall lifetime AB use: macrolide use during the first 2 years of life (past exposure/current exposure)	Dichotomous (more than 2 courses of macrolides in the first 2 years of life vs. non-exposed)	National database on prescription drug purchase	OR
17	No	Definite asthma by 7 years of age: medical records	Birth child/7 years of age (implicitly)	Prevalence (future prevalence/current prevalence)	Maternal AB exposure: AB by oral or injection during 4 weeks prior to last menstrual period or at any month during pregnancy (current exposure/past exposure)	6 categories of AB subdivided in 5 categories for timing of exposure (total exposure, 4 week before LMP, 1st trimester, 2nd trimester and 3rd trimester)	Questionnaires during pregnancy	OR

Nr.	Do the authors mention "(study) design" in the text (+ location)	Case (event or state) definition for asthma	Scientific T_0 (explicitly/implicitly mentioned + temporal aspects)	Measure of disease occurrence (+ abstract temporal aspects)	Exposure conceptualization (+ abstract temporal aspect)	Operationalization of the exposure	Measurement method exposure	Measure of association
18	Yes (discussion & introduction)	Treated asthma: at least 2 prescriptions at different time points within a 12 months period, of anti-asthmatic drugs 'Current' asthma at age 6 years or older and at age 13 years or older	At 13 months of age (implicitly)	Incidence rate ratio (future incidence)	AB exposure during the first 12 months of life: prescription of at least 1 antibiotic (current exposure)	Dichotomous, AB ever (Yes/No) Number of courses (4 categories) Penicillins (Yes/No) Cephalosporins (Yes/No) Macrolides (Yes/No) Other drugs (Yes/No)	Drug prescription records	Incidence rate ratio's
19	No	Prevalence of asthma: ever been diagnosed with asthma	Inclusion in study (implicitly)	Prevalence (current prevalence)	AB exposure in the first year of life (past exposure)	Dichotomous (Yes/no)	Modified version of the ISAAC questionnaire	OR
20	Yes (discussion)	Asthma: Asthma diagnosis by year 3 (ever having asthma) (diagnosis by a physician, parent-reported)	At 1 year of age (implicitly)	Incidence (future incidence)	Systemic AB us: antibiotic use during pregnancy (current exposure)	Prenatal AB use: dichotomous (yes/no) (any AB during pregnancy) Timing of AB use (first trimester vs. second and third trimester)	Questionnaires	OR
21	Yes (abstract, introduction, methods & discussion)	Asthma: Diagnosis of asthma and 1 or both: at least 2 dispensed asthma drugs with gaps of at least 2 weeks between distributions or at least 3 dispensed asthma drugs or short acting β_2-agonists within a year	At 1 year of age (implicitly)	Prevalence (future prevalence) Incidence (future incidence)	Filled prescriptions of systemic antibiotics: any AB, airway AB and urinary tract or skin and soft tissue AB (during pregnancy and in the first year of life) (current exposure)	Any antibiotics in fetal life: dichotomous (Yes/No) Per trimester (1,2 and 3) Idem for airway AB and urinary tract/skin AB	Swedish prescribed drug register	HR
22	Yes (discussion)	Incident asthmatic patients between age 2-8 years (doctor's diagnosis)	Asthma onset (implicitly)	Incidence (current incidence)	History of AB consumption in the first year of life History of AB consumption during pregnancy (past exposure)	AB use in the first year of life, dichotomous (Yes/No) Exposure to AB during pregnancy, dichotomous (Yes/No)	ISAAC questionnaire	OR
23	No	Diagnosed asthma: received reimbursement for medication for asthma, after 3 years of age Asthma age 3-5 Asthma age 6 and older (current prevalence)	6 months prior to asthma occurrence (explicitly)	/ (current prevalence)	Prenatal AB exposure Postnatal AB exposure (from birth until asthma diagnosis, excluding use 6 months before diagnosis) (first year of life, during the first 3 years of life) (past exposure)	Group of AB (7 categories) Indication of AB (3 categories)	Drug prescription register	OR

Nr.	Do the authors mention "(study) design" in the text (+ location)	Case (event or state) definition for asthma	Scientific T_0 (explicitly/implicitly mentioned + temporal aspects)	Measure of disease occurrence (+ abstract temporal aspects)	Exposure conceptualization (+ abstract temporal aspect)	Operationalization of the exposure	Measurement method exposure	Measure of association
24	Yes [methods (under funding) & introduction]	Severe wheeze or asthma exacerbation: receipt of oral corticosteroid for 3 or more days, admission to hospital, or emergency room because of asthma or wheeze needing treatment with oral steroids	First AB prescription (explicitly)	/ (future incidence)	AB prescription: age in days when the child was prescribed the first AB (current exposure)	Dichotomous (Yes/No) Age of AB prescription (4 categories)	Medical records	HR
25	Yes (methods)	Childhood asthma in a 14 year period 1.Recurrent hospital admissions for asthma 2. Long-term outpatient attendance related to asthma 3. Recurrent use of inhaled corticosteroids (collected at pharmacy)	Asthma occurrence (implicitly)	Incidence rate (current incidence)	AB in pregnancy AB 80 weeks before pregnancy AB 80 weeks post-partum (past exposure)	Dichotomous (Yes/No) for: Any AB, AB RTI, AB UTI, AB other infection, AB 80-40 weeks before pregnancy, AB 40 weeks before pregnancy to pregnancy, AB pregnancy, AB birth to 40 weeks post-partum, AB 40-80 weeks post-partum	National prescription registry	Incidence rate ratio's
26	No	Asthma at age 91 months: maternal report of a physician diagnosis of asthma at any time and symptoms of wheezing in the previous 12 months.	At 2 years of age (implicitly)	/ (future prevalence)	Any AB use during 0-24 months Total number of times AB had been taken between 0-24 months Timing of AB intake (current exposure)	Dichotomous (any vs. none) Testing dose dependency (5 levels) Testing time dependency (8 levels)	Questionnaires	OR
27	Yes (introduction)	Asthma: at least 1 inpatient claim record or 2 ambulatory claims (2-6 years)	At 1 year of age (implicitly)	Incidence rate (future incidence)	Exposure to acetaminophen and/or AB in the first year of life	Categorical: Non-exposure, only AB, only acetaminophen, both Number of AB prescriptions first year of life (4 categories) (current exposure)	Prescription claims data	HR
28	No	Asthma ever: child ever having an asthma attack Current asthma: at least 1 wheezing episode in the last 12 months or at least 1 night of cough attack not related to infection	Asthma occurrence (implicitly)	Prevalence (current prevalence)	AB use in the first year of life (any) (past exposure)	Dichotomous (Yes/No)	ISAAC questionnaire	OR
29	No	'Current' doctor diagnosed asthma at 8 years of age: doctor's diagnosis and either or both 'current' asthma medication and current wheezing (last 12 months) Non-atopic asthma = 'current' asthma and not having allergic sensitization, or 'current' doctor's diagnosis of rhino conjunctivitis, food allergy or eczema	At 1 year of age (implicitly)	/ (future prevalence)	Treatment with broad-spectrum AB during the first week of life (current exposure)	Dichotomous (Yes/No)	Questionnaire at 6 months of age	OR
30	No	Symptoms of asthma: ever diagnosed with asthma	Asthma occurrence (implicitly)	Prevalence Incidence (current prevalence)	Use of AB in the first year of life Use of AB between 13 and 36 months of age (past exposure)	In 5 categories (never, 1-2 times, 3-4 times, 5-6 times and more than 6 times)	Questionnaire based on ISAAC and ECRHS II	OR

Nr.	Do the authors mention "(study) design" in the text (+ location)	Case (event or state) definition for asthma	Scientific T_0 (explicitly/implicitly mentioned + temporal aspects)	Measure of disease occurrence (+ abstract temporal aspects)	Exposure conceptualization (+ abstract temporal aspect)	Operationalization of the exposure	Measurement method exposure	Measure of association
31	Yes (abstract, methods & discussion)	COPSAC: acute severe asthma exacerbations (need for oral prednisolone, high-dose ICS or asthma hospitalization) (at least 1 exacerbation in the first 5 years; at least 1 hospitalization in the first 5 years) DNBC: Asthma hospitalization Asthma medication (at least 1 ICS prescription)	Birth child (implicitly)	/ (future incidence)	COPSAC: maternal use of AB in the third trimester of pregnancy DNBC: maternal use of AB during pregnancy (at least 1 prescription filled during the period defined as date of birth minus gestational age in days) (current exposure)	Dichotomous (Yes/No)	Questionnaires	HR
32	No	Asthma diagnosis ever 'Current' asthma	Inclusion at age 9-12 years (implicitly)	Prevalence (current prevalence)	AB use during infancy (past exposure)	Dichotomous (Yes/No)	Questionnaire	OR
33	Yes (introduction, methods & discussion)	Asthma medication: asthma (event): any medication for asthma, except oral β2-agonists, dispensed at least twice	Date of first exposure to antibiotics (explicitly)	Incidence Incidence rate (future incidence)	Prescription of AB: at least once between birth and end of follow-up (current exposure)	Groups of antibiotics (3 categories, with each 4 subgroups)	The prescribed drug register	HR
34	No	Physician diagnosis of asthma Asthma-like symptoms: cough without wheeze for at least 3 weeks or wheeze after playing at least 2 different episodes	Asthma occurrence (implicitly)	Prevalence rate (current prevalence)	AB consumption during the first year of the child's life (past exposure)	Dichotomous (Yes/No)	Questionnaire based on ISAAC phase III core and environmental questionnaires	OR
35	Yes (discussion)	Medical diagnosis of asthma in 5 year olds	4-5 years of age (implicitly)	Incidence (current incidence)	Postnatal exposure to various classes of AB (past exposure)	AB doses per type of AB at 4-5 years of age	Questionnaire	OR
36	No	Asthma diagnosis ever: the lifetime prevalence of asthma diagnosis Current asthma: Last 12 months prevalence of asthma treatment Asthma treatment, last 12 months: prevalence of current asthma (lifetime diagnosis + wheezing 12 months of survey)	Inclusion in study (implicitly)	Prevalence (current prevalence)	Use of AB during infancy for more than 3 days (past exposure)	Dichotomous (Yes/No)	Modified ISAAC questionnaire	OR
37	No	Physician diagnosis of asthma after 6 months of age with history of wheezing in the 6th year of life (current asthma)	At 6 months of age (implicitly)	/ (future prevalence)	AB exposure before 6 months of age Number of AB courses before 6 months of age (current exposure)	Dichotomous (Yes/No) Number of courses (3 categories)	Interview with mother at child's 6th birthday	OR
38	Yes (discussion)	Asthma: prevalence of asthma at age 4 and 8 years: more than 3 episodes of wheeze or at least 1 episode of wheeze combined with inhaled glucocorticoids for asthma or asthma symptoms in the previous 12 months	At 6 months of age (implicitly)	Prevalence (future prevalence)	AB use during the first year of life (ever AB) (current exposure)	Dichotomous (Yes/No)	Questionnaire at 1 year	OR

Nr.	Do the authors mention "(study) design" in the text (+ location)	Case (event or state) definition for asthma	Scientific T_0 (explicitly/implicitly mentioned + temporal aspects)	Measure of disease occurrence (+ abstract temporal aspects)	Exposure conceptualization (+ abstract temporal aspect)	Operationalization of the exposure	Measurement method exposure	Measure of association
39	Yes (methods)	Ever asthma (active MD asthma): active MD-asthma was reported at least once on questionnaires at age 1, 2, 3 and/or 5 (diagnosed by a physician as having asthma and who were reported to have had active symptoms during the past year)	At 9 months of age (implicitly)	Prevalence (future incidence)	Use of systemic AB in the first 9 months of life (current exposure)	Ever and per year (first 5 years of life)	Nurse interview at 2 weeks, 1 month, 2, 3, 4, 6 and 9 months of age	OR
40	No	Diagnosis of asthma ever Treatment of asthma in the last 12 months	Inclusion study (implicitly)	Prevalence (current prevalence)	Occurrence of AB use during infancy (past exposure)	/	Questionnaires (written and video)	OR
41	No	Asthma: prevalence of asthma in children and young adults (7-23 years)	End of the neonatal period (implicitly)	Prevalence (future prevalence)	AB therapy: in the neonatal period: at least 4 intravenous doses of penicillin and an aminoglycoside in neonatal period without having an infection (current exposure)	Dichotomous (Yes/No)	Hospital records	OR
42	No	Severe asthma symptoms: ('current') wheezing causing sleep disturbance, or wheezing limiting speech, or at least 4 attacks of wheezing in the past 12 months Asthma ever	6-7 years of age (implicitly)	/ (current prevalence)	AB use in the first year of life (past exposure)	Dichotomous (Yes/No)	ISAAC questionnaire	Prevalence odds ratio's
43	No	Wheeze in the last 12 months (6-7 years, current wheeze)	6-7 years of age (implicitly)	Prevalence (current prevalence)	AB in the first year of life (past exposure)	Dichotomous (Yes/No)	ISAAC questionnaire at 6-7 years	OR
44	Yes (discussion)	Asthma diagnosis: hospital discharge for asthma or 2 medical fee-for-service claims within a moving 12-months period or 2 prescriptions for a known asthma medication within a moving 12-months period	At 1 year of age (implicitly)	Incidence (future incidence)	Exposure to AB in the first year of life (current exposure)	Dichotomous (ever/never) Number of AB prescriptions (4 levels) Type of antibiotic dispensed (6 categories)	Administrative health data (database)	HR Incidence rate asthma
45	No	'Current' asthma: doctor's diagnosis of asthma & current wheeze between 4-5 years of age Atopic 'current' asthma: current asthma + positive SPT at 5 years or elevated serum total IgE at 5 years	At 1 year of age (implicitly)	/ (future prevalence)	AB use in the first year of life (current exposure)	Categorical (AB use 0-6 months, 7-12 months and 1-12 months)	Daily diary	OR
46	Yes (methods)	Childhood asthma: at least 1 diagnosis of asthma and at least 1 prescription for asthma medication within a 2-year period	First occurrence of an asthma diagnosis (code) and a filled prescription for asthma medication within a 2 year period: refer to this as index date (explicitly)	Incidence (current incidence)	Mean number of AB prescriptions filled per month during pregnancy AB in the first 6 months of life or AB prior to index date (past exposure)	Dichotomous for AB use child (Yes/No) Not clear how they coded the variable for the mother	From administrative linked health databases	Rate ratio's OR

Nr.	Do the authors mention "(study) design" in the text (+ location)	Case (event or state) definition for asthma	Scientific T_0 (explicitly/implicitly mentioned + temporal aspects)	Measure of disease occurrence (+ abstract temporal aspects)	Exposure conceptualization (+ abstract temporal aspect)	Operationalization of the exposure	Measurement method exposure	Measure of association
47	No	'Current' asthma: asthma + wheeze and/or inhaler use occurring in the previous 12 months	Asthma diagnosis period (implicitly)	Prevalence (current prevalence)	AB use: ever in the first 3 months AB use: between 3 and 15 months (past exposure)	3 categories: systemic AB use, 1 chest infection, 2+ chest infections at 3 months, 15 months and 4 years	Questionnaire at 3 and 15 months of age	OR
48	Yes (methods & discussion)	'Current' wheeze (past 12 months): asthma at 3.5 and 7 years of age	At 1 year of age Asthma onset (at 3.5 or 7 years of age) (implicitly)	Prevalence (future prevalence/current prevalence)	AB in the first year of life (current exposure/past exposure)	Dichotomous (Yes/No)	Questionnaire at 3.5 years of age	OR
49	Yes (introduction)	'Current' asthma at age 7: at least 2 physician visits for asthma, 1 asthma hospitalization or 2 prescriptions for any asthma drug in the year following the 7th birthday	At 1 year of age/ At 7 years of age (implicitly)	Prevalence (future prevalence/current prevalence)	AB use during the first year of life (number of oral AB prescriptions) (current exposure/past exposure)	Number of oral AB prescriptions (4 categories)	Healthcare administrative data	OR
50	No	Cumulative prevalence of asthma (wheezing ever) Current prevalence of asthma (wheezing last 12 months)	6-7 years of age (implicitly)	Cumulative prevalence Current prevalence (current prevalence)	AB use in the first year of life (past exposure)	Dichotomous (Yes/No)	ISAAC questionnaire at age 6-7 years old	OR
51	Yes (discussion & methods)	Doctor's diagnosis of asthma: ever asthma diagnosis	5-13 years of age/1 year of age (implicitly)	Prevalence (current prevalence/future prevalence)	Use of AB in the first year of life (past exposure/current exposure)	Never/first use after 12 months of age and first use first 12 months of age	Parental questionnaire among children age 5-13 years	OR
52	No	Asthma: 6-7 years and 13-14 years	Inclusion in study 6-7 years and 13-14 years (implicitly)	Prevalence (current prevalence)	AB use in the first year of life (past exposure)	/	ISAAC questionnaire at age 6-7 years and 13-14 years	OR
53	No	Prevalence of asthma symptoms	6-7 years of age (implicitly)	Prevalence (current prevalence)	AB use in the first year of life (past exposure)	Dichotomous (Yes/No)	ISAAC questionnaire (telephone + written)	Prevalence OR
54	No	Asthma at age 2 (1-2 years) Asthma at age 5 (2-5 and active 4-5 years)	At 1 year of age (implicitly)	/ (future prevalence)	Number of courses of AB (oral) in the first year of life (current exposure)	Categorical (number of courses in 4 categories)	Automated pharmacy records (prescriptions dispensed at outpatient pharmacies in the clinical centers)	OR
55	No	Prevalence of symptoms of asthma: asthma ever	Inclusion in study: 13-14 years of age (implicitly)	Prevalence (current prevalence)	Total AB-sales: defined daily doses (average daily dose used) (past exposure?)	Defined daily doses for total AB, broad spectrum AB and narrow spectrum AB	Obtained from Institute for Medical Statistics	Regression coefficient

Nr.	Do the authors mention "(study) design" in the text (+ location)	Case (event or state) definition for asthma	Scientific T_0 (explicitly/implicitly mentioned + temporal aspects)	Measure of disease occurrence (+ abstract temporal aspects)	Exposure conceptualization (+ abstract temporal aspect)	Operationalization of the exposure	Measurement method exposure	Measure of association
56	No	Self-reported (diagnosis) of asthma	At 5 years of age (implicitly)	Prevalence (future cumulative incidence, prevalence?)	AB prescription first 5 years of life (current exposure)	Per class of AB (7categories) Per age of prescription up to 5 years (5 categories) For both also the total number of prescriptions	General practitioner records	OR
57	No	Incident diagnosis of asthma	At birth (implicitly)	Incidence (future incidence)	Prescribed AB during pregnancy (current exposure)	4 categories of total number of prescriptions	General practice research database	HR
58	No	Asthma at age 5: doctor's diagnosis of asthma + at least 1 episode of wheezing in the previous 12 months	At 1 year of age (implicitly)	/ (future prevalence)	Courses of oral AB in the first year of life (current exposure)	Categorical (3 categories)	Telephone questionnaire from birth to age 5	OR
59	No	Incident diagnoses of asthma: incidence of disease after the age of 1 year	At 6 months of age, at 1 year of age and at 2 years of age? (implicitly)	Incidence (future incidence)	AB prescriptions in the first year of life (current exposure)	In classes of AB (5 categories) Number of prescriptions (6 categories)	Extraction from large longitudinal primary care dataset (information collected as part of routine	HR
60	Yes (methods)	Asthma: ever asthma	At 1 year of age (implicitly)	Prevalence (future prevalence)	AB in the first year of life (current exposure)	Dichotomous (Yes/No)	Questionnaire parents when child was 7-8 years old	OR
61	Yes (methods & discussion)	Asthma: lifetime prevalence (doctor's diagnosis at least once or more than 1 doctor's diagnosis of asthmatic, spastic or obstructive bronchitis)	Inclusion in study (implicitly)	Lifetime prevalence (current prevalence)	AB courses in the first 3 years of life (past exposure)	Number of AB courses (4 categories)	Retrospective through questionnaires at age 5 7 years and 9-11 years	Prevalence OR
62	No	History of asthma: (ever asthma)	Inclusion in study at 5-10 years of age (implicitly)	Prevalence (current prevalence)	AB use: AB ever AB use in the first year of life (past exposure)	Dichotomous (Yes/No) 3 categories (AB use first year/after first year, only/never)	Questionnaire children 5-10 years	Prevalence OR
63	No	Asthma: recurrent episodes of wheeze after the age of 2 years	At 12 months of age (implicitly)	Cumulative incidence (future incidence)	Treatment with oral AB in the first 2 years of life (current exposure)	Dichotomous (Yes/No, when looking for an association with asthma)	Medical records GP	OR

Part 3 of the table with all extracted information from the reviewed articles:

Nr.	Confounders (+ abstract temporal aspects)	Effect modifiers (+ abstract temporal aspects)	Justification selection confounders and/or effect modifiers	Domain of the study	Design of data collection	Design of data processing	Referral to reporting guideline or theoretical work?
1	Maternal age at delivery; maternal education level; child's age; child's gender; gestational age at birth; delivery mode; feeding status first 6 months of life (exclusive breast feeding: yes/no); family history (allergic disorders: yes/no/unknown)	Child's age; child's gender; feeding status first 6 months of life (exclusive breast feeding: yes/no); family history (allergic disorders: yes/no/unknown)	Yes: previous publications	Children 6 months of age	Case-control study Hospital-based case-control study Retrospective study	Multiple logistic regression	No
2	Age; gender; number of days on which healthcare providers were visited (not specified when); income (insurance premium at onset asthma or before the last day of 2015); place of residence (at onset asthma or before the last day of 2015)	/	No	Children and adolescents	Cross-sectional study	Multiple logistic regression	No
3	Complete set of confounders not specified, table 1 and table 3 include possibly a couple of confounders: rhinitis (ever); rhinitis (12 months before onset asthma); 'rhinoconjuntivitis' (in the last 12 months); eczema (yes/no, first 12 months of life); traffic next to house (not specified); family member smoking at home (during the first year of life); wheezing chest after exercise (last 12 months); dry cough at night (last 12 months)	Complete set of effect modifiers not specified, table 1 and table 3 include possibly a couple of effect modifiers: rhinitis (ever); rhinitis (12 months before onset asthma); 'rhinoconjuntivitis' (in the last 12 months); eczema (yes/no, last 12 months); acetaminophen use (yes/no, first 12 months of life); traffic next to house (not specified); family member smoking at home (during the first year of life); wheezing chest after exercise (last 12 months); dry cough at night (last 12 months)	Explanation on how confounding was checked in statistical analysis section	Children 6-13 years old	Cross-sectional study	Poisson regression Multivariable analysis	No
4	Residence location (at birth); length of gestation (at birth); number of siblings (at birth); SES (at birth); infant gender; maternal asthma (at birth child); postnatal AB exposure first year of life	Infant gender; method of birth; newborn feeding method	No	Children	Retrospective cohort study Population-based cohort study	Time-to-event analysis Cox regression	No
5	Urban residence (not specified); allergic rhinitis (not specified); atopic dermatitis (not specified); chronic rhinitis (not specified); age; acute sinusitis (not specified); gastroesophageal reflux (not specified); use of NSAIDs 120 days before asthma onset; gender; subtype of antibiotics (first 2 years of life)	Age-stratified analysis	No	Children with a history of acute bronchiolitis in the first 2 years of life	Matched case-control study	Conditional logistic regression	No
6	Prematurity; gender; caesarean delivery; other drug classes (first 6 months of life)	Any significant first order interaction terms; interaction with time	Yes: publication on taking into account interaction with time	Children 6 months of age	Retrospective cohort analysis Retrospective cohort study	Cox proportional hazards regression	No
7	Gender; familial factors (by sibling analysis)	/	Yes: referred to studies using the same design to adjust for confounding	Children 1 year of age	Retrospective study Retrospective cohort study	Cox proportional hazards regression	No
8	Maternal smoking during pregnancy; being born at <37 weeks gestation; any breast feeding for 4 months or longer; parental levels of education; parental asthma; being born small for gestational age; caesarean section; doctors-diagnosis of food allergy (first year of life); parental rhinitis, parental eczema; gender; maternal medication (during pregnancy); recurrent wheeze (first year of life); introduction of egg before 9 months of age; introduction of fish before 9 months of age; fish once a month or more in infancy; damp mould in the home (at 6 months of age); cat at home during infancy	/	No	Children 12 years of age/children 1 year of age	Prospective birth cohort study Follow-up study	Multivariate logistic regression	No

Nr.	Confounders (+ abstract temporal aspects)	Effect modifiers (+ abstract temporal aspects)	Justification selection confounders and/or effect modifiers	Domain of the study	Design of data collection	Design of data processing	Referral to reporting guideline or theoretical work?
9	Maternal history of allergy; maternal age at pregnancy; maternal smoking during pregnancy; mode of delivery; gestational age at delivery; day-care attendance (during the first 2 years of life); number of previous live births; bronchitis (at 2 years of age); gender	/	No	Children at 2 years of age	Hospital-based prospective birth cohort study	Multivariate logistic regression	No
10	Generation R, Pacman and Breathe: age; gender; family history of asthma or allergy Seaton: gender; family history of asthma or allergy	Age-stratified analysis	No, based on available variables in the database	Children 1 year of age (Generation R) Children 6 months of age (Seaton) Children 3 years of age (Pacman and Breathe)	2 population based cohorts 2 asthma cohorts	Multivariable logistic regression Meta-analysis	No
11	Matched for age and gender; maternal history of asthma; vaginal bleeding during pregnancy; maternal age ≥ 30; exclusive breastfeeding (neonatally)	/	No	Children aged 7-14 years/newborns	Retrospective case-control study	Forward logistic regression Multivariate regression analysis	No
12	Age; gender; parental history of allergy; paracetamol use first year of life; bronchiolitis first year of life; consumption of unpasteurized milk (first year of life)	Rural residence in the first year of life	No	Children aged 6 to 7 years/children 1 year of age	Cross-sectional study	Multivariate logistic regression Multivariable analysis	No
13	Parent reported infections (first year of life); eczema (overall); age; household income (unspecified); number of siblings (current); child care (first 5 years of life); race; sex; pets (first year of life); parental atopy; breast feeding	/	No	Children aged 0-21 years of age	Cross-sectional family-based study	Poisson regression model	No
14	Gender; age at delivery; use of acid-suppressive drugs (during pregnancy); use of drugs indicated for allergic dermatitis (during pregnancy); use of drugs indicated for allergic rhinitis (during pregnancy); use of insulin (during pregnancy); potential time trends (in the case-sibling analysis); child birth order; use of asthma medication (during pregnancy); use of antidepressants (during pregnancy)	/	Yes: publication	Preschool children	Case-sibling study Nested case-sibling study Case-sibling analysis Case-control study Case-control analysis Matched case-control design	Conditional logistic regression	No
15	Maternal smoking during pregnancy; maternal asthma status; maternal age at delivery; maternal educational level; gestational age at delivery; infant's birth hospitalization length of stay; birth weight; infant's race; gender; having chronic lung disease (not specified); having congenital heart disease (not specified); type of most severe bronchiolitis healthcare encounters experienced during infancy; birth year; study site	Subgroup analysis: birth weight, KPNC or Tenn Care	Yes: covariates chosen a priori based on clinical relevance	children 1 year of age/children aged 4.5 to 6 years	Population-based birth cohort study	Multivariable logistic regression model	/

Nr.	Confounders (+ abstract temporal aspects)	Effect modifiers (+ abstract temporal aspects)	Justification selection confounders and/or effect modifiers	Domain of the study	Design of data collection	Design of data processing	Referral to reporting guideline or theoretical work?
16	/	/	No	Children aged 2-7 years/children 2 years of age	Cohort	Fisher's test	No
17	Maternal age at delivery; race (mother); number of previous births; smoking during pregnancy; maternal drug allergy history; married at pregnancy; educational level; maternal asthma history	Stratification by time of medication (not explicitly)	Yes: previous publication	Newborns/children up to the age of 7 years	Prospective cohort study?	Multilevel multiple logistic regression	No
18	Year of birth; maternal age at birth; gestational age; type of delivery; sex; mother's formal education; birth weight; RTI requiring hospital admission during the first 12 months of life (confounding by indication)	/	No	Children 1 year of age	Population-based birth cohort study	Poisson models	No
19	Age (at inclusion); BMI (at inclusion); household income (at inclusion); parental history of allergic disease; sex; school area; ETS (at inclusion)	Stratification by genotype TLR4: modifies combined effect of infant AB exposure in the first year of life and history of physician diagnosed bronchiolitis in the first 2 years of life on the development of asthma	No	Children	Cross-sectional study	Logistic regression	No
20	Study intervention; maternal history of asthma; maternal ethnicity (Mexican ancestry); ibuprofen use during pregnancy; maternal age; smoking during pregnancy; exposure to smoke in the home in the first year of life; child antibiotic use for respiratory reasons; vitamin use of the mother during pregnancy; any ibuprofen use during the child's first year of life; any respiratory infections during the child's first year of life	Vitamin use of the mother during pregnancy; maternal history of asthma; any ibuprofen use of the child in the first year of life; children who did not use AB in the first year of life; respiratory infections in the child's first year of life; smoking in the home during the child's first year of life	No	At-risk children 1 year of age	Cohort Prospective study	Multivariable logistic regression	No
21	Maternal smoking during pregnancy, parity (at delivery), family situation, mother's country of birth, sex, gestational age, respiratory diagnoses as newborn, maternal age at delivery, maternal AB exposure in fetal life, AB exposure defined from hospital diagnoses (unspecified), parental education, maternal asthma, age as analysis time scale, birth weight, mode of delivery	Interaction birth order and exposure; interaction term parity and age	Yes: refer to a study adjusting for familial factors by using sibling control analysis	Children 1 year of age	Nationwide population-based register study Nationwide population-based birth cohort	Cox proportional hazards regression	No
22	Maternal smoking (past); history of asthma or atopic diseases in first- and second-degree relatives; number of older or younger siblings in the household; preterm delivery; duration of breast feeding; delivery method	Interaction AB consumption and other asthma risk factors not significant	Yes: referral to previous publications	Children 2 to 8 years of age	Case-control study	Multiple logistic regression	No
23	Previous deliveries; maternal use of antibiotics during pregnancy; maternal asthma; gestational age; total number of AB purchases	Maternal age; smoking during pregnancy; previous deliveries; mode of delivery; ponderal index child; maternal asthma; previous miscarriages; gestational age; maternal background and perinatal factors; maternal or child's use of AB	Yes: referral to own previous publication	Children	Population- and register-based study Population- and register-based nested case-control study	Conditional logistic regression	No
24	Sex; attendance day-care; older siblings; breast feeding	Modification of effect of AB by age; interaction AB prescription and atopy	No	Children	Population-based birth cohort Longitudinal analysis	Cox models	No
25	Calendar year; age; birth weight; multiple births; maternal age; maternal smoking during pregnancy; maternal asthma; parity; mode of delivery; sex; season of birth; maternal employment	Stratification by maternal asthma; stratification by treatment indication	No, but report that they have chosen the confounders a priori and refer to the appendix for more Information	Children	Prospective registry-based cohort study	Log-linear Poisson regression models	No
26	Sex; ethnicity; age of child at outcome; marital status (unspecified); home ownership status (unspecified); mother's highest educational qualification; degree of difficulty in paying for food (unspecified); mother's age at time of delivery; birth mode; birth weight; gestation; breast feeding (first year of life); time spent outdoors (child) (unspecified); disinfectant use mother during pregnancy (hygiene); smoking mother during pregnancy; child's contact with cats (at 24 months)	Interaction term exposure and sex	No referral but in text: based on prior reports or theoretical grounds	Children 2 years of age	Population-based cohort Longitudinal cohort Longitudinal birth cohort	Logistic regression	No

Nr.	Confounders (+ abstract temporal aspects)	Effect modifiers (+ abstract temporal aspects)	Justification selection confounders and/or effect modifiers	Domain of the study	Design of data collection	Design of data processing	Referral to reporting guideline or theoretical work?
27	Gender; geographical area at birth; inpatient visits (during the first year of life); bronchitis diagnosis (during the first year of life); enrolee category at birth; number of ambulatory visits (during the first year of life); otitis media diagnosis (during the first year of life)	/	No	Children 2 years of age	Prospective birth cohorts Birth cohort	Cox proportional hazard	No
28	Children's age; family history of asthma and rhinitis; prematurity; children's sex	/	No	Children	Cross-sectional study	Multinomial logistic regression	No
29	Maternal smoking during pregnancy; any breast feeding for 4 months or more; parental level of education; atopic heredity; gender; maternal medication during pregnancy; gestational age; doctor's diagnosis of food allergy during the first year of life; eczema during the first year of life; fish at least once per month at 1 year of age; fermented food at least once per month at 1 year of age; choice of spread at 1 year of age; daily outdoor activity at 12 months; father employment at 6 months; rural living at 6 months; caesarean section; small for gestational age; admission to neonatal ward; fish before 9 months of age	Interaction term: atopic heredity and neonatal AB treatment/ parental asthma and neonatal AB treatment/ early fish introduction and neonatal AB treatment	No, but explained what confounders they took into account	Children 1 year of age (not explicitly reported)	Prospective cohort study	Multivariate logistic regression	No
30	Sex was a statistically significant factor in all models	Sex was a statistically significant factor in all models	No	Children aged 6-8 years	Questionnaire study	Multivariate binary logistic regression	No
31	COPSAC: sex; paracetamol use (during pregnancy); following up to 5 years of age DNBC: sex; age; siblings; parental asthma; maternal age at delivery; cow's milk in diet child (unspecified); day care (unspecified); pets (unspecified); maternal income and educational level (unspecified); population density; type of birth; gestational age; birth weight; breast feeding (first year of life); asthma seasonality; maternal eczema and allergic rhinitis; maternal use of tobacco (during pregnancy), alcohol, paracetamol and exercise during pregnancy; paternal education	COPSAC: / DNBC: sex; age; interactions sex and age; subgroup analysis by sex and type of AB	Yes: previous publications	COPSAC: newborns of mothers with a history of asthma DNBC: newborns up to the age of 5 years	COPSAC: clinical birth cohort study DNBC: cohort study design	Cox proportional hazards	No
32	Sex; history of atopic dermatitis (mother and father); income (unspecified); age; ETS (unspecified); BMI child	/	No, only report that they predefined confounders to adjust for	Children aged 9-12 years	Cross-sectional study	Logistic regression	No
33	Birth month	Gender; interaction exposure and age	No	Children	Cohort Population based cohort study Register-based cohort study	Cox proportional hazards regression	No
34	/	/	No	Children aged 3-6 years	Cross-sectional study	Chi-square test and McNemar's test	No
35	Number of respiratory episodes (first 5 years of life); child's gender; prenatal and postnatal (4-5 years of age) ETS; maternal age and education; AB doses; maternal atopy; parity	Interaction term macrolide use and cephalosporins	No, but a priori chosen	Children 5 years of age	Prospective cohort study Prospective observational study	Multivariate logistic regression	No
36	Age; BMI; degree maternal education; sex; parental asthma; second-hand smoking (current)	/	No	Children	/	Logistic regression	No

Nr.	Confounders (+ abstract temporal aspects)	Effect modifiers (+ abstract temporal aspects)	Justification selection confounders and/or effect modifiers	Domain of the study	Design of data collection	Design of data processing	Referral to reporting guideline or theoretical work?
37	Infant's/child's race; number in household (at 6 years of age); passive smoking in pregnancy; alcohol exposure in pregnancy; maternal hypertension (during pregnancy); preterm labor; resuscitation at birth; admitted to NICU; length of NICU stay; mechanical ventilation in NICU; neonatal sepsis or bacteremia; neonatal meningitis; breast feeding; history of parental asthma; LRTI during the first year of life; maternal parity; household income (during pregnancy)	History of parental asthma; LRTIs during the first year of life.	No, but explained how these were selected (likelihood ratio test)	Children 6 months of age	Observational cohort	Logistic regression	No
38	Sex; older siblings; parental allergy; pneumonia first year; otitis first year; young maternal age; maternal smoking (unspecified); exclusive breast feeding; bronchitis first year	Interaction AB use and respiratory infections (during the first year of life); subgroup analysis (with and without allergic signs first year of life)	No	Children 6 months of age	Birth cohort study	Logistic regression	No
39	Confounding by indication (number of visits to physician in the first 9 months of life); gender; feeding status (infant)	Stratification for AB use; combined variable including AB use and number of illness visits (in the first 9 months of life).	No	Children 9 months of age	Prospective study	Logistic regression	No
40	/	/	No	Children 6-12 years of age and 12-15 years of age	Cross-sectional questionnaire-based survey (ISAAC)	Multiple logistic regression	No
41	Gestational age; parental smoking and level of education (first year of life); sex; number of older siblings; birth weight; mode of delivery; year of birth	/	No	Children	Prospective cohort study	Logistic regression	No
42	Sex; language; maternal education; ever breast fed; current diet (at 6-7 years of age); region (not specified); gross national income (not specified); paracetamol use first year of life; parental smoking (not specified); siblings (not specified)	/	No	Children aged 6-7 years	Cross-sectional study	Generalized linear mixed models Multiple regression analysis	No
43	Gender; school decile (at 6-7 years of age); maternal farm animal exposure during pregnancy; born in New Zealand; number of older siblings (at 6-7 years of age); number of younger siblings (at 6-7 years of age); contact farm animals in first year; paracetamol use in first year; current paternal and maternal smoking (at 6-7 years of age); cooking appliance (at 6-7 years of age); television viewing (at 6-7 years of age); ethnicity; maternal education (at 6-7 years of age); breast feeding (first year of life); maternal smoking first year; cat at home in the first year; dog at home in the first year; food intake (at 6-7 years of age); truck passage street (at 6-7 years of age); physical activity (at 6-7 years of age)	/	No	Children aged 6-7 years	Cross-sectional survey	Logistic regression	No
44	Gender; SES at birth; urban or rural address at birth; birth weight; gestational age; delivery method; frequency of physician visits (first year of life); allergist/respirologist/immunologist visit (first year of life); hospital visit involving surgery (first year of life); congenital anomalies (first year of life); related diseases (otitis media/bronchitis/URTI and LRTI; first year of life)	/	No	Children 1 year of age	Longitudinal cohort study Observational prospective cohort study	Cox proportional hazard	No
45	Propensity score; number of doctor's visits; sex; childcare (first year of life); pets (first year of life)	Number of doctor's visits (first year of life); sex; childcare (first year of life); pets (first year of life); AB use between 0-6 months	No	Children at 1 year of age and at high risk for atopy	High-risk birth cohort Prospective birth cohort	Propensity score adjustment Logistic regression	No

Nr.	Confounders (+ abstract temporal aspects)	Effect modifiers (+ abstract temporal aspects)	Justification selection confounders and/or effect modifiers	Domain of the study	Design of data collection	Design of data processing	Referral to reporting guideline or theoretical work?
46	Age at diagnosis; gender; allergic disease prior to index date; administration of oxygen to newborn in hospital; one or more diagnoses of bronchopulmonary disease (prior to index date); maternal receipt of social welfare (in year before or during pregnancy); intranasal corticosteroids during pregnancy; paternal history of asthma; presence of wood-burning fireplace in home (prior to index date); day-care attendance (prior to index date); pets in the home (for more than 2 months) (prior to index date); breast feeding; asthma during pregnancy; asthma siblings (prior to index date)	/	No	Children up to the age of 10 years	Case-control study, nested in a cohort of children Two-stage case-control study	Conditional logistic regression	No
47	Number of chest infections (0-4 years of age); gender; prioritized ethnicity; family history of asthma, eczema/hay fever; AB use during outcome period; household smoking (any by father or mother); ear infection (0-4 years of age); parity (before 3 months of age)	Number of chest infections (0-4 years of age)	No	Children	Prospective study Birth cohort study Cohort	Cross-sectional associations Multivariate logistic regression	No
48	Age; smoking (pregnancy); day care or looked after by others (first year of life); presence of dog (first year of life); used pillow (first year of life); used quilt (first year of life); slept on used mattress (first year of life); hospitalized (first year of life); asthma in parents (current); maternal smoking (current); eczema in child (current); rhinitis in child (current); positive skin test in child (current); gender; SGA (at birth); first born (at birth); SES (at birth); damp patches on walls in winter (first year of life); fungus/mould all year (first year of life); presence of cat (first year of life); presence of bird (first year of life); slept on sheepskin (first year of life); children in household (at 1 year); duration of breast feeding (first year of life); carpet in bedroom (first year of life); trucks passing house frequently (7 years of age)	Interaction with age and significant factors: presence of dog (first year of life); maternal smoking during pregnancy; used quilt (first year of life); slept on used mattress (first year of life); hospitalization (first year of life); parental asthma; eczema in child (current); rhinitis in child (current); positive skin test in child (current); current maternal smoking; day care or looked after by others (first year of life)	No	Children 1 year of age/children aged 3.5 and 7 years	Prospective study	Generalized linear models Logistic regression	No
49	Gender; maternal history of asthma; number of health care visits (first year of life); number of non-respiratory tract infections (unspecified); number of siblings (at 7 years of age); urban/rural location (unspecified); number of LRTI (unspecified); household income (unspecified)	Maternal history of asthma; presence or absence of dog during birth year; urban/rural location (unspecified)	No	Children 1 year of age/children 7 years of age	Longitudinal study Cohort	Multivariate logistic regression	No
50	/	/	No	Children (6-7 years of age and 13-14 years of age)	Cross-sectional survey	Logistic regression using the forward conditional method	No
51	Age; sex; country; maternal smoking during pregnancy; maternal asthma and/or rhinoconjunctivis; paternal asthma and/or rhinoconjunctivis; having household pets during the first year of life; use of antipyretics during the first year of life; measles infection (not specified); type of diet (not specified); MMR vaccination; current smoking in the household; older siblings; parental education	/	Yes: referral to previous results in other publication	Children aged 5-13 years/children 1 year of age	Cross-sectional	Logistic regression	No
52	/	/	No	Children aged 6-7 and 13-14 years old	Cross-sectional survey School-based survey Questionnaire based study	Multivariate analysis: unconditional logistic regression	No
53	Dataset; sex; family size; paracetamol use (first year of life)	Stratification for type of infection (first year of life)	No	Children aged 6-7 years old	Asthma prevalence study	Maentel-Henszel procedure Logistic regression	No

Nr.	Confounders (+ abstract temporal aspects)	Effect modifiers (+ abstract temporal aspects)	Justification selection confounders and/or effect modifiers	Domain of the study	Design of data collection	Design of data processing	Referral to reporting guideline or theoretical work?
54	Sex; number of office visits to a health care practitioner (in the first year of life); number of illnesses of the respiratory tract in the first year of life (upper and lower).	Stratification for eczema (discussion)	No	Children 1 year of age	Longitudinal follow up Cohort	Multivariate logistic regression	No
55	Level of westernization or affluence of a country (gross national product)	/	No, but report that they correct for the confounders because they have been shown to be associated with asthma	Schoolchildren aged 13-14 years old	Ecological analysis	Linear regression	No
56	Age; sex; parental allergic disease; social class (current, adulthood); number of siblings (current)	Stratification for age at prescription	No	Children 5 years of age	Retrospective cohort study Cohort study	Logistic regression	No
57	Gender; parental allergic disease; general practice; AB first 6 months of life; child's consulting behaviour in first 6 months of life in quartiles; maternal allergic disease; parental smoking; maternal age; year of birth	/	No	Newborns	Birth cohort	Cox regression	No
58	Child's race; day care attendance first year of life; in utero exposure to smoking; number of months of breast feeding; paternal history of hay fever; maternal hay fever; maternal eczema; average number of cigarettes per day smoked by all adults in the household (unspecified); sex; annual household income (unspecified); paternal history of asthma; paternal history of eczema; maternal asthma; number of older siblings	/	Yes: refer to prior published results	Children 1 year of age	Prospective birth cohort study	Logistic regression	No
59	Sex; parental allergic disease; parental smoking (unspecified); consulting behaviour (first year of life); impact of asthma severity variable (in the year following diagnosis of asthma)	/	No	Children 6 months of age/children 1 year of age/children 2 years of age	Birth cohort study Cohort	Cox regression	No
60	Gender; residential area (current); parental asthma; parental hay fever; older siblings; maternal smoking during pregnancy; ETS during first 6 months of life; history of LRTI during first 2 years of life	Stratification for parental hay fever	No	Children 1 year of age	Cross-sectional	Multiple logistic regression	No
61	Family history of atopy; parental education; school grade; number of siblings; study area; sex	Family history of atopy; number of siblings; sex	No	Children aged 5-7 years and 9-11 years	Cross-sectional	Multivariate logistic regression	No
62	Age; ethnicity; family history of asthma; mother and father's current smoking; smoking mother and father during child's first year of life; gender; family size; eczema (ever); hay fever (ever)	/	No	Children 5-10 years of age	Cross-sectional study Retrospective study	Logistic regression	No
63	Year of birth; number of visits to the family doctor (between 0-5 years of age)	Interactions for model	No	Children 1 year of age	Retrospective study	Stepwise Logistic regression	No

7.3 Appendix 3: Critical review of the extracted information – examples

Reporting of outcome, exposure and the domain in the aim

Example 1 refers to an article that reported outcome, exposure and the domain in the aim and example 2 two refers to one that did not. More specifically, example 2 only reported to have studied the prevalence of asthma, while in the text of the article it appeared that they also assessed whether antibiotic use is a risk factor for asthma.

Example 1:

"…to evaluate the hypothesis that exposure to either acid-suppressive medications or ABX during infancy is associated with an increased risk of childhood allergic disease."

Example 2:

"… to study the current prevalence 12 years later in an inner-city group of children between 6 and 13 years old. Our hypothesis is that similar to other Latin American countries the prevalence of both asthma and allergic rhinitis in Costa Rica has increased."

Reporting of the measure of occurrence

Forty-five out of 63 articles reported the measure of occurrence (prevalence or incidence of asthma). Example 3 refers to an article that reported the measure of occurrence and example 4 to one that did not. Example 3 refers to the primary outcome being the incidence of disease after the age of 1 year, while example 4 does not refer to the measure of occurrence.

Example 3:

"…antibiotics during the first year of life would affect the subsequent incidence of allergic disease. …antibiotic prescriptions in the first year of life as the primary exposures and incidence of disease after the age of 1 year as the primary outcome."

Example 4:

"The primary outcome in this analysis was physician-diagnosed asthma after 6 months of age with history of wheezing in the sixth year of life."

Reporting of the case (event or state) definition

Out of 63 articles, 59 reported a case (event or state) definition. Example 5 refers to an article that reported the case definition and example 6 to one that did not. More specifically, example 5 refers to an article where the asthma case definition was derived from a questionnaire at 5 years of age asking about the previous 12 months. Example 6 refers to an article where the authors did not report on how asthma was assessed and what the case definition for asthma was.

Example 5:

"Asthma current: A positive answer from the caregiver to the question (child at 5 y old), "Has your child ever been diagnosed by a doctor as having asthma in the past 12 months?"

Example 6:

"Neonatal sepsis and antibiotic therapy were considered as exposures and asthma, hay fever and atopic eczema as outcomes."

Reporting of the conceptualization and operationalization of the exposure

Fifty-seven out of 63 articles reported the conceptualization and operationalization of the exposure. Example 7 refers to an article that reported the conceptualization and the operationalization of the exposure and example 8 to one that did not. More specifically, example 7 refers to 'any use of antibiotics in the first year of life', which implies that antibiotic use in the first year of life was operationalized as 'any use' vs 'no use'. Example 8 on the other hand refers to an article where the exposure to antibiotics during pregnancy was only reported in the abstract and in the results tables. In the methods section, the authors only reported that they extracted information necessary for the study without further details.

Example 7:

"Information on any use of antibiotics in the first year of life, …, was obtained from the core questionnaire, which was supplemented with questions on socio-demographic and household characteristics, history of allergic diseases, and the living environment."

Example 8:

"After obtaining consent from the parents, maternal data sheets on pregnancy and delivery were sourced from the hospitals where the children were delivered, and the information required for the present study was extracted and evaluated."

Temporal relation between outcome and exposure

For 50 articles, the temporal relation between the outcome and the exposure could be derived from the aim or the case (event or state) definition and conceptualization of the exposure. Example 9 refers to an article where the temporal relation between outcome and exposure could be derived and example 10 refers to one where this temporal relation could not be derived. More specifically, example 9 refers to an article where the authors indicated the temporal relation between the outcome and exposure, i.e. asthma occurrence was assessed at 12 years of age and exposure to antibiotics was assessed in the first week of life. Example 10 refers to an article where the authors only reported that they investigated the association between antibiotic use and asthma, but they did not specify when exposure and outcome measurement took place in time. As a consequence, the temporal relation could not be derived for example 10.

Example 9:

"…to examine the prevalence of, and risk factors for, asthma at 12 years of age and to examine the associations with atopic asthma and nonatopic asthma. In particular, we wanted to analyse whether the long-term effects of antibiotics during the first week of life could be seen at the age of 12 years."

Example 10:

"We investigated key hygiene factors, including, antibiotic use, …, and their association with food allergy and asthma in the same cohort of patients in which many showed comorbidity…."

Reporting of confounders considered and effect modifiers taken into account

Regarding confounders and effect modifiers, 56 out of 63 articles reported the confounders that were considered and only 24 out of 63 articles reported to have taken effect modifiers into account. Since no further explanation is necessary, no examples are provided.

Reporting of the domain of the study

All articles reported the domain of the study. However, none of the authors explicitly referred to this as the 'domain'. Example 11 shows how articles implicitly refer to a domain. The domain for this example is children 6-7 years of age.

Example 11:

"We have therefore investigated the association between infections at age 0-4 years, medication use (antibiotics and paracetamol) early in life, and the subsequent risk of childhood asthma at age 6-7 years."

7.4 Appendix 4: Derivation of the 'theoretical design' – examples

Consensus on 'theoretical design' being 'the current occurrence of asthma as a function of past exposure to antibiotics':

Example 12 shows an article where there was consensus from the start about the 'theoretical design' being the 'current occurrence (incidence) of asthma onset as a function of past exposure to antibiotics (maternal antibiotic consumption during pregnancy or child antibiotic consumption in the first year of life) in children 2 to 8 years of age.' Asthmatic children between the age of 2 and 8 were diagnosed and the exposure to antibiotics during the first year of life was reconstructed. T_0 can be considered here to be asthma onset and all confounders adjusted for were either fixed or measured at T_0 (maternal smoking history and the number of older or younger siblings).

Example 12:

Aim of the study: *"…the primary objective was to investigate the association between antibiotic exposure and the risk of developing childhood asthma."*

Measure of occurrence (and abstract temporal aspect): (current) incidence (*"To recruit incident asthmatic patients, we adopted the Global Initiative for Asthma (GINA) criteria prospectively between March and September 2010."*)

Case (event or state) definition: Incident asthmatic patients (between the age of 2 and 8 years)

Conceptualization and operationalization of the exposure (and abstract temporal aspect): History of antibiotic consumption in the first year of life; history of antibiotic consumption during pregnancy (past exposure); (*"…data related to the history of antibiotic consumption in the first year of life and during pregnancy were collected by interviewing the parents/guardians."*)

Confounders (and abstract temporal aspects): Maternal smoking history (measured at T_0); history of asthma or atopic diseases in first- and second-degree relatives (fixed); number of older or younger siblings in the household

(measured at T_0); preterm delivery (fixed); duration of breast feeding (fixed); delivery method (fixed)

Effect modifiers (and abstract temporal aspects): Interaction between antibiotic consumption and other asthma risk factors (unspecified)

Scientific T_0: at asthma onset

Domain of the study: Children 2-8 years of age

Design of data collection: *"This investigation was a case-control study conducted between March and September 2010 in Urmia, Iran."*

Design of data processing: *"We fitted multiple logistic regression models…"*

Consensus on the 'theoretical design' being the 'future occurrence of asthma as a function of current exposure to antibiotics':

Example 13 shows an article where there was consensus from the start about the 'theoretical design' being the 'future occurrence (incidence) of asthma onset as a function of current exposure to antibiotics (use of systemic antibiotics in the first 9 months of life) in children 9 months of age. A physician-diagnosis of asthma was assessed in children up to the age of 5 years and asthma occurrence was compared among exposed and non-exposed. Exposure was assessed in the first 9 months of life and confounders taken into account were also assessed during the first 9 months of life. T_0 was at 9 months of age.

Example 13:

Aim of the study: *"…we examined the association between oral antibiotic use in the first 9 months of life and the development of physician-diagnosed asthma, physician-diagnosed eczema, and allergic allergen sensitization by 5 years of age in a non-selected cohort followed prospectively from birth…."*

Measure of occurrence (and abstract temporal aspect): (future) prevalence (incidence?); (*"Fisher's exact test was also used to assess relations of early antibiotic use to active asthma, eczema, and specific allergen sensitization at age 1, 2, 3, and 5 years, and to prevalence of ever asthma, eczema, and sensitization by age 5."*)

Case (event or state) definition: ever asthma (= active MD asthma: diagnosed by a physician as having asthma and active symptoms during the past year)

Conceptualization and operationalization of the exposure (and abstract temporal aspect): Systemic antibiotic use in the first 9 months of life (current exposure); (*"Children were considered to have used systemic antibiotics in the first 9 months of life if parents reported use of oral antibiotics at any interview."*)

Confounders (and abstract temporal aspects): Confounding by indication (number of visits to physician in the first 9 months of life); gender (fixed); feeding status (infant)

Effect modifiers (and abstract temporal aspects): Combined variable including antibiotic use and number of illness visits (in the first 9 months of life).

Scientific T_0: at 9 months of age

Domain of the study: children 9 months of age

Design of data collection: ***"****The Infant Immune Study (IIS) is a prospective birth cohort study of the immunologic and exposure predictors of the development of asthma and allergic disease in childhood."*

Design of data processing: "*Fisher's exact test … to assess relations of early antibiotic use to active asthma… and to prevalence of ever asthma,…. The relation of number of courses of antibiotics to ever asthma was assessed by trend chi-square. Odds ratios were calculated using logistic regression …."*

No consensus initially on 'theoretical design':

Example 14 shows an article where first there was disagreement about the 'theoretical design', but after discussion agreement was reached about the 'theoretical design' probably being 'the future occurrence (cumulative incidence, prevalence?) of asthma onset as a function of current exposure (treatment with oral antibiotics in the first two years of life) in children'. For this example it was not clear which confounders were adjusted for and whether they were measured after or at T_0 (at 2 years of age).

Example 14:

Aim of the study: *"…investigate the putative relationship between childhood infections and atopic disorders further, we conducted a detailed temporal analysis of family doctor recorded diagnoses of childhood infections, including … diagnoses of asthma, … for a 1975–84 birth group at a single general practice."*

Measure of occurrence (and abstract temporal aspect): (future) cumulative incidence (prevalence?); (*"All subjects were followed up to at least 12 years of age, thereby encompassing the peak incidence of atopic disorders (fig 1)."*)

Case (event or state) definition: asthma (recurrent episodes of wheeze after the age of two years)

Conceptualization and operationalization of the exposure (and abstract temporal aspect): Treatment with antibiotics in the first two years of life (current exposure); (*"We found no relationship between the number of older siblings and the number of episodes of any infection, nor with antibiotic treatment in the first two years of life (table 2)."*)

Confounders (and abstract temporal aspects): year of birth (fixed); number of visits to the family doctor (between 0-5 years of age).

Effect modifiers (and abstract temporal aspects): interactions for model (unspecified)

Scientific T_0: At 2 years of age

Domain of the study: children at 2 years of age

Design of data collection: *"In this retrospective study three variables significantly predicted subsequent asthma, hay fever and eczema—namely, maternal atopy, immunization with whole-cell pertussis vaccine, and treatment with oral antibiotics in early life."*

Design of data processing: "*Stepwise logistic regression analysis was performed incorporating all the medical and social variables studied including social class, year of birth, and number of visits to the family doctor in early childhood (p for inclusion <0.2, p for exclusion > 0.4, to include weak effects)."*

8 Appendices chapter 3

8.1 Appendix 1

Contacting the authors

An online questionnaire was designed and sent out to the corresponding authors of the selected articles (cf. infra, the search strategy for the selection of articles is described elsewhere[6]). The questionnaire was designed in Qualtrics software, version December 2019 (Copyright 2019 Qualtrics, Provo, Utah, USA, cf. infra).

The corresponding authors were contacted via the e-mail address provided in the articles. In case no corresponding author was indicated in the article, the first author was contacted.

The e-mail included a link to the questionnaire and an accompanying information letter. This information letter explained the context for performing this study and the aim of the study. Authors were also provided with information on theoretical design with reference to relevant textbooks (cf. infra).

In case of no response after the first e-mail, two reminders were sent out (each one after two weeks). If the e-mail could not be delivered to the corresponding author (because of an invalid e-mail address), the last or first author was contacted depending on who the corresponding author was.

Response

In total, 15 authors completed the questionnaire. Out of these 15 authors, 7 were first author of the article as well as the corresponding author and 4 were last author of the article and also corresponding author. Of the remaining 4 authors, one author was the last author and was contacted by us because the e-mail address of the corresponding (and first) author was invalid, and three authors were last and corresponding author but forwarded the questionnaire to the first author of the article who then filled in the questionnaire. For one author we decided to omit the questionnaire because the author requested not to quote the answers.

Selected articles

For the following articles (n = 30) the corresponding author was contacted or the first author in case no corresponding author was indicated in the article.

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Information letter

Theoretical design in epidemiological (etiological) research

Context

Our research department, Epidemiology and Social Medicine (ESOC), has been involved in asthma research since 1990. We have been one of the participating centers in the European Community Respiratory Health Study (ECRHS) and in the International Study of Asthma and Allergies in Childhood (ISAAC). The department also started its own birth cohort, the Prospective study on the Influence of Perinatal factors on the Occurrence of asthma and allergies (PIPO). Specific interest goes to the influence of early life exposures on the occurrence of allergic diseases in childhood, including the association between exposure to antibiotics in early childhood and the occurrence of asthma[10]. Up to date, results of research on this association have been conflicting. We want to investigate to what extent these conflicting results can be explained by differences in the approach of study design. To our knowledge, only a few reviews focus on methodology, but mostly restricted to the method of data collection (retrospective vs prospective) and the influence of confounding by indication and reverse causation[7-9]

Aim

One of the aims of this PhD project is to examine the influence of theoretical design on results in epidemiological etiologic research. The first part of this project is a

methodological review. We critically reviewed papers, that investigated the causal relationship between exposure to antibiotics and the occurrence of asthma, on the reported study design with emphasis on theoretical design. Your paper was one of the papers that was reviewed. Therefore, we would like to ask you 17 questions concerning your study design.

Theoretical design

Our interest goes in particular to the formulation of a theoretical design. Theoretical design is an important part of study design in epidemiological research as described by Grobbee and Hoes in their textbook on clinical epidemiology[1]. According to the textbook, this starts with the formulation of a research question, which should include the exposure(s), the outcome and the domain. This research question is then translated into an occurrence relation. The occurrence relation in etiologic research is the outcome as a function of the exposure, taking other relevant determinants into account (extraneous: confounders; non-extraneous: effect modifiers)[1]. A similar approach was already described in the 80's of last century by Olli Miettinen, who also includes the time relation and directionality as important elements of the occurrence relation[2]. Also David Kleinbaum emphasized that this directionality is crucial for the interpretation of epidemiological findings, since it allows the researcher to distinguish between what is an antecedent and what is a consequence.[10]

Review of the papers

We extracted the following information relating to study design from the selected papers:

- Research question or aim
- The domain of the research
- Definition of the outcome and exposure and the measure of outcome occurrence
- Reported study design: the theoretical design, the design of data collection & the design of data analysis (with special interest for the temporal aspects, the measure of association and the addressment of confounders and effect modifiers.)

In case of non-explicit formulation of a theoretical design, we defined ourselves a theoretical design based on the information from the article.

The extracted information and the formulated theoretical designs was registered at the Institutional Repository of the University of Antwerp (IRUA) on the 4th of December 2019. After this date no changes were made to this document.

Questions to the authors

The next step in our project is to ask the authors of the papers about different aspects of theoretical design related to their published research. We do not have the intention to question authors about their knowledge on this subject. We are interested in how the authors initially conceived the theoretical design of their study before going into the reviewing process, because we are aware that the reviewing process could lead to changes in the manuscript.

We have formulated 17 questions and we hope that you are willing to answer them. This will only take 5 to 10 minutes of your time. We will then compare the formulated answers with the registered table. The results of this exercise will be communicated with the authors, with the view of receiving comments and will be presented for publication to the European Journal of Epidemiology. Feel free to contact us after filling in the questionnaire if you would like to consult the theoretical design we have formulated for your study. We are happy to share this information with you.

Thank you for your participation!

Contact:

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Promotor: prof. dr. Joost Weyler - Social Epidemiology and Health Policy, department of Epidemiology and Social Medicine, Faculty of Medicine and Health Sciences, University of Antwerp – joost.weyler@uantwerp.be

Questionnaire (online survey)

Dear researcher,

In the following sections of this survey we will ask you questions that relate to the study design of your article that was sent to you as attachment in the mail you have received. Answering these questions will only take 5 to 10 minutes of your time.

We will first ask for your first and last name and the title of your article. We need this information to be able to connect the provided information with the correct article and with the theoretical design(s) we have formulated ourselves.

During the survey you are able to close your browser. Your answers will be saved automatically and upon your return you can continue the survey where you left off. If you have additional questions please do not hesitate to contact us: hayat.bentouhami@uantwerpen.be. At the end of the survey we also give you the opportunity to provide us with comments.

We thank you for your participation!

Best regards

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Please indicate that you understand that we use your first name and last name to connect the provided information and the correct article reviewed and the theoretical design we have formulated and that you are willing to participate in this survey.

- o I understand and agree
- o I do not wish to fill in the survey

Please enter your first and last name.

Please enter the title of your article that was reviewed by us.

1. According to you, what is the research question behind your study?

2. What is the domain of your study?

3. Was the primary aim of your study to investigate a causal relationship between antibiotics exposure and the occurrence of asthma, or was this a secondary aim?
    - Primary aim
    - Secondary aim

4. What is the translation, according to you, of your research question into an occurrence relation (theoretical design)?

5. How did you sample the study population?
    - Cumulative sampling
    - Case-cohort sampling
    - Incidence density sampling
    - Other (specify):

6. Can you specify what the directionality is between outcome (asthma) and exposure (antibiotics)?
    - The current prevalence of asthma as a function of past exposure to antibiotics.
    - The current incidence of asthma as a function of past exposure to antibiotics.
    - The current prevalence of asthma as a function of current exposure to antibiotics.
    - The current incidence of asthma as a function of current exposure to antibiotics.
    - The future prevalence of asthma as a function of current exposure to antibiotics.
    - The future incidence of asthma as a function of current exposure to antibiotics.
    - Other (specify):

7. Have you used any aspect of some causal theory in order to decide on confounders and effect modifiers (e.g.: DAGs)?
    - No
    - Yes (specify which theory):

8. What are the confounders you have taken into account to adjust for the crude association between exposure to antibiotics and the occurrence of asthma?

9. Have you considered effect modification in your analysis for the association between exposure to antibiotics and the occurrence of asthma?
    - No
    - Yes (specify which effect modifiers):

10. How familiar are you with the concept of theoretical design?
    - Not at all familiar
    - Slightly familiar
    - Somewhat familiar
    - Moderately familiar
    - Extremely familiar

11. How familiar are you with the concept of causal theory (e.g. DAGs,…)?
    - Not at all familiar
    - Slightly familiar
    - Somewhat familiar
    - Moderately familiar
    - Extremely familiar

12. How familiar are you with the concept of confounding?
    - Not at all familiar
    - Slightly familiar
    - Somewhat familiar
    - Moderately familiar
    - Extremely familiar

13. How familiar are you with the concept of effect modification?
    - Not at all familiar
    - Slightly familiar
    - Somewhat familiar

- Moderately familiar
- Extremely familiar

14. Did you make changes to your manuscript that you did not agree with, but the reviewers expected you to do so?
    - No (go to question 16)
    - Yes (go to question 15)

15. If yes, where these changes related to:
    - The formulation of the research question or aim
    - The case and control definition
    - The statistical analysis
    - Adjustment for confounding
    - The handling of effect modification
    - Other (specify):

16. Was there any comment of one of the reviewers that you did not agree with, and if so what comment was that (please also send us the comments of the reviewers from the first reviewing process to hayat.bentouhami@uantwerp.be)?
    - I agreed with all the comments.
    - I did not agree with the following comment(s):

17. Was there an epidemiologist involved in your study?
    - No
    - Yes

If you have any additional comments, please state below:

End of questionnaire

8.2 Appendix 2

Answers of the authors

Below, the answers of the authors are presented one by one per group and per topic. The answers are quoted verbatim and are anonymized.

Research question

The authors were asked to formulate a research question. In the information letter accompanying the questionnaire, we explained that a research question should encompass the outcome, the exposure, as well as the domain of the study.

Group 1:

1. To assess the association between use of antibiotic during the first 3 years of life and the risk of developing childhood asthma and the occurrence of asthma exacerbations.
2. To determine the association between oral antibiotic use in the first year of life and asthma at age 7 years in a large cohort of children
3. What is the association between antibiotic use during pregnancy and the occurrence of asthma in preschool children.

Group 2:

4. evaluate whether exposure to antibiotics during the first 12 months of life increases the risk of subsequent treated asthma (identified through antiasthmatic drug prescriptions) in a large population-based birth cohort retrospectively followed for up to 17 years, with a carefully planned design capable of decreasing the chance of bias.
5. What is the current prevalence of asthma, allergic rhinitis, and eczema related symptoms in school-aged children in Costa Rica?
6. Our study was to examine whether antibiotic use affected the risk of atopic diseases (current wheeze or allergic rhinoconjunctivitis) differently between children living in urban areas in early life and those living in rural areas in early life
7. to identify and quantify risk factors for asthma
8. My basic research question is how enviornment is neccessary for healthy development in children.
9. the relation between postnatal antibiotic exposures within the first 2 years of life and allergic diseases in children at 5 years of age in Japan

Group 3:

10. To identify the determinants of the incidence of childhood asthma
11. To determine the relative importance and cumulative effect of pre- and post-natal environmental risk factors on the development of childhood asthma"
12. Does the use of antibiotics prenatally have an association with asthma and/or wheezing in young children?
13. Does use of medications that disturb the microbiome in infancy increase subsequent risk of developing allergic diseases?

Group 4:

14. Observational study of AB use and its impact on te microbiome and health in early life

A research question encompassing exposure, outcome and the domain of the study was formulated by one author in group 1 (number 3, although forgetting the question mark at the end of the sentence). This was also the case for one author in group 2 (number 9). Number 9 also reported the location where the study was performed, which is not relevant in the research question of a study. Another author in group 2 (number 5) formulated a question, but did not encompass all three elements. Also this author reports the location where the study was performed. In group 3, one author (number 12) formulated a research question encompassing all three elements. Another author in group 3 (number 13) formulated a research question but did not encompass the domain of the study.

All other authors reported the aim or the objective of the study instead of a research question. Number 4 also includes in the answer the way the data were collected for the study and number 14 also describes the type of study that was performed.

Theoretical design

The authors were asked to indicate the domain of their study, to translate the research question into an occurrence function and to indicate the directionality (select one of the presented occurrence functions) for their study.

<u>Domain</u>

Group 1:

1. Children
2. Human epidemiologic study (but I'm not 100% clear on your meaning of domain)
3. Pregnant women (population-based).

Group 2:

4. Not sure about the question
5. Respiratory Epidemiology in children
6. The domain was children aged 6 to 7 years who were enrolled in school and resided in the city of San Francisco (province of Córdoba, Argentina) and the surrounding rural areas that have a similar climate, and dairy farm activities and grain harvests (soya, corn, and wheat) are common.
7. A cross-sectional survey of 10 873 6–7-year-old children in Auckland, Bay of Plenty, Nelson and Christchurch
8. Epidemiology and Public Health
9. pediatric allergy, birth cohort

Group 3:

10. Observational study in asthma
11. I am not quite get the question itself
12. I'm not sure what this means. Clinical research?
13. allergy

Group 4:

14. Human health & microbiology

In group 1, one author (number 1) reported the domain of the study. In group 2, one author (number 6) reported the domain. This author also specifies the location where the study population was selected from. This location does not represent the domain, but only the location where participants were recruited in order to sample within the domain of the study. For two authors (number 5 and 7) in group 2, the domain could be deduced from the answer. Number 5 also indicates the area of research which is not relevant for the specification of the domain. Number 7 also specifies the type of survey carried out and the location where the study population was selected, but both are not relevant for the specification of the domain.

The other authors did not specify the domain of their study, neither could it be deduced from their answers. In group 1, one author (number 3) refers to the population in which exposure was measured (as the domain) instead of the population in which the outcome was measured. Five other authors reported the area or topic of research instead of the domain (numbers 8 and 9 in group 2, numbers 10 and 13 in group 3 and number 14 in group 4). The remaining authors (number 2 in group 1, number 4 in group 2 and numbers 11-12 in group 3) reported that they were not sure about the meaning of domain or did not understand the question. Number 2 explains what type of study they performed in general terms (an epidemiological study in humans). Number 12 asked whether 'domain' could be the type of research.

<u>Occurrence function</u>

Group 1:

1. The use of antibiotic therapy early in life influences the risk of developing asthma through the immunomodulatory effect of antibiotic on the immune system, which leads to imbalanced Th1/Th2. Although, it is also possible that children who are in need of antibiotic early in life have already a Th1/Th2 imbalanced caused by the infections treated with the antibiotic and that this makes them more susceptible for asthma later in life.
2. Birth cohort design
3. Not sure what you mean here. Asthma in preschool children as a function of antibiotic use in pregnant women, independent of risk covariates.

Group 2:

4. Not sure
5. none. this is simply and observation but to casual.
6. Antibiotic use in the first year of life was associated with current wheeze and allergic rhinoconjunctivitis among school-aged children who lived in an urban area of Córdoba (Argentina) early in their lives, but not among children who lived in a rural area early in their lives. This results suggested that rural residence in early life may protect against detrimental effects of antibiotic use on allergic diseases among children."
7. Don't understand the question
8. I am not sure of the meaning of your question, I always think that the research question could be applied to basic epidemiological design (e.g. whether we could know the causal association of our research question by cohort study).
9. Allergic diseases such as asthma, atopic dermatitis, and allergic rhinitis are common chronic diseases around the world and have led to a greater economic burden in health care costs. Antibiotic prescriptions were still made on a large scale in Japan, with 60% of patients prescribed antibiotics for upper respiratory infections in 2009.

Group 3:

10. I do not understand this question
11. Again, I am not familiar with the terminology here. I am not sure how to address your question
12. I'm not looking at a causal relationship since this is a prospective cohort study, not a randomized controlled trial. I'm looking at an association. I'm not sure what an occurrence relation or theoretical design is. Theoretically, antibiotics may impair the immune system in developing fetus, supporting the hygiene hypothesis.
13. We found a statistically significant association between use of antibiotics and antacid medications during the first 6 months of infancy and subsequent development of allergic disease. As a retrospective study, were identified association but cannot conclude causation.

Group 4:

14. This was an observational trial as alternatives are not considered ethical. Hence, so no causality can be inferred. However, a causal relation between antibiotic use and antibiotic resistance was inferred. We observed an association between antibiotic use (of notably macrolides) and disturbed microbiota, BMI-z and asthma (at 5 years).

Only one author (number 3 in group 1) formulated the occurrence function of their study, even though the author was not completely sure about the meaning of the question.

All other authors did not formulate an occurrence function. In group 1, number 1 explained the rationale for performing the study and the biological pathway for the association studied. Also number 9 in group 2 reports the rationale for performing the study. Some authors (number 6 in group 2, number 13 in group 3 and number 14 in group 4) summarized the results of their study instead of formulating an occurrence function. Number 13 adds to the answer that they could not conclude causation because they performed a retrospective study. Number 14 reports the type of study that was performed and also states that they could not infer causality because they

conducted an observational trial. Nevertheless, they inferred a causal relation between antibiotic use and antibiotic resistance. Author number 2 in group 1 only reports the type of study that was performed. One author (number 5 in group 2) states that there is no occurrence function for their study and describes their study as an 'observation' and not causal. However, in the article the authors did investigate the relationship between antibiotic use and the occurrence of asthma. The other remaining authors (numbers 4, 7 and 8 in group 2 and numbers 10-12 in group 3) indicated that they were not sure about the question or did not understand it. Number 12 (in group 3) states that they did not look at a causal relationship because they have performed a prospective cohort study and not a randomized controlled trial (RCT).

<u>Directionality between outcome (asthma) and exposure (antibiotics) (occurrence function)</u>

Group 1:

1. The current incidence of asthma as a function of current exposure to antibiotics.
2. The future prevalence of asthma as a function of current exposure to antibiotics.
3. The current incidence of asthma as a function of past exposure to antibiotics.

Group 2:

4. The current incidence of asthma as a function of past exposure to antibiotics.
5. The current prevalence of asthma as a function of past exposure to antibiotics.
6. The current prevalence of asthma as a function of past exposure to antibiotics.
7. The current prevalence of asthma as a function of past exposure to antibiotics.
8. The current incidence of asthma as a function of past exposure to antibiotics.
9. The current prevalence of asthma as a function of past exposure to antibiotics.

Group 3

10. The current incidence of asthma as a function of past exposure to antibiotics.
11. The current prevalence of asthma as a function of past exposure to antibiotics.
12. The current incidence of asthma as a function of past exposure to antibiotics.
13. Other: Technically we used incidence density as a function of past exposure to antibiotics since time of follow-up varied per child.

Group 4:

14. The future incidence of asthma as a function of current exposure to antibiotics.

In group 1, the directionality selected by number 3 in the questionnaire was concordant with the directionality deduced from the article. This was also the case for number 5 and 7 in group 2 and for number 10 in group 3.

For four articles the directionality could not be deduced from the information in the article (number 2 in group 1, number 6 in group 2, number 11 in group 3 and number 14 in group 4). Number 2 and 14 selected in the questionnaire to have investigated the 'future occurrence of asthma as a function of current exposure to antibiotics'. Number

6 and 11 selected in the questionnaire to have investigated the 'current occurrence of asthma as a function of past exposure to antibiotics'.

For all other authors, the selected directionality in the questionnaire was not in concordance with the directionality that was deduced from the information in the article. Number 1 in group 1 indicates that the directionality is cross-sectional. For numbers 4 and 9 in group 2 HB and JW extracted from the article that the directionality was the 'future occurrence of asthma as a function of current exposure to antibiotics' because the authors report in the article to have performed a '(prospective) cohort study'. In the questionnaire, number 9 reports to have sampled the study population as the general population from a birth cohort. Number 4 reports in the questionnaire to have included all records from administrative data and to have performed a population-based birth cohort study. For number 8 in group 2, the directionality deduced from the article was 'future incidence of asthma as a function of current exposure to antibiotics'. In the article the authors reported to have performed a retrospective cohort study and to have used Cox proportional hazards to estimate a hazard ratio. In the questionnaire however, the author reports to have sampled the study population by cumulative sampling (which is a sampling method in 'nested case-control' studies). For number 12 in group 3, the directionality deduced from the information in the article was 'future incidence of asthma as a function of current exposure to antibiotics', because the authors reported in the article to have performed a cohort/prospective study. Also in the questionnaire, the author reports to have performed a 'prospective cohort study'.

Causal theory

The authors were asked whether they used any aspect of causal theory to select confounders and/or effect modifiers and if so, which aspect of causal theory.

Group 1:

2. Bradford Hill criteria with confounding factors identified in the literature. This study was conducted in the early 2000s prior to the popular use of DAG.
3. DAG

In group 1, two authors reported to have used some aspect of causal theory to select confounders and or effect modifiers. Number 2 reports in the questionnaire to have used the Bradford Hill criteria with confounding factors identified in literature for the selection of confounders. This information was not reported in the article. Number 3 reports in the questionnaire to have used directed acyclic graphs (DAGs), but in the article the authors reported to have used previous publications for the selection of confounders and/or effect modifiers.

In group 1, 2 and 3 all other authors indicated to not have used any aspect of causal theory to select confounders and/or effect modifiers, even though all report to have

adjusted for confounding in the article. Of these, four authors report in the article some justification for selecting confounders and effect modifiers (numbers 5 and 8 in group 2 and numbers 11 and 13 in group 3), albeit it not being some aspect of causal theory (e.g. selection of confounders based on previous publications).

The author in group 4 (number 14) indicated to not have used any aspect of causal theory, neither in the questionnaire nor in the article. In the study (according to what was reported in the article) no confounders or effect modifiers were taken into account. In the questionnaire however, the author does indicate to have adjusted for age development.

Confounding

The authors were asked which confounders they adjusted for.

All authors reported to be moderately to extremely knowledgeable with the concept of confounding. The majority of the authors reported the same information on adjustment for confounding in the questionnaire and in the article.

In group 2 there was one author (number 10) that reported in the questionnaire to not have adjusted for confounding because they were not looking at causal relationships. In the article however, a set of confounders adjusted for was reported. In group 4, number 14 reported in the questionnaire to have adjusted for age development, while this was not reported in the article.

Effect modification

The authors were asked whether they took into account effect modification and if so, which effect modifiers.

The majority of the authors reported to be moderately to extremely knowledgeable with the concept of effect modification. Among these authors, three did not consistently report the same information on taking into account effect modification in the questionnaire and in the article (number 3 of group 1 and numbers 11 and 13 of group 3):

One author reported not to be knowledgeable at all with the concept of effect modification (number 14 in group 4) and another author reported to be somewhat knowledgeable with this concept (number 9 in group 2).

9 Appendices chapter 4

9.1 Additional file 1

Design of data processing – additional information

Multiple imputation of missing data

Missing data were imputed by applying Multiple Imputation by Chained Equations (MICE) under the assumption that missing data are missing at random (MAR).[35] Data were not missing completely at random (MCAR), because missingness was associated with some of the characteristics of the population moments and the events. The number of imputations was arbitrarily set at 20 (29.4% of the children had a missing value for at least one of the included variables, but there was no difference between setting the number of imputations at 20 or more). In the imputation model, all variables of interest for the model with missing data (i.e.: parental education, breast feeding for at least 6 months, day-care attendance, LRTIs in the first year of life, paracetamol (acetaminophen) use in the first year of life, ETS, parental asthma and atopic dermatitis) were included. Additionally (to improve the precision of the imputation), auxiliary variables (variables not included in the model, but associated with missingness) were included in the imputation (i.e.: delivery method, the presence of older siblings, wheezing during the first year of life and exposure to pets). For imputation, the logistic regression method was used since all variables with missing data are binary variables.

9.2 Additional file 2

Association between (first) asthma occurrence and antecedents of exposure to systemic antibiotics in the first year of life: complete case approach (CCA)

The main findings from the multiple logistic regression models in the CCA are presented in supplementary table 1. In the CCA, the crude odds ratio contrasting events and population moments as an estimator for the crude incidence density ratio (IDR) comparing 'exposed' with 'unexposed' was 2.24 (95% CI 0.93, 5.38), although not statistically significant at the α-level of 0.05. Possible effect modification by sex, parental education, ETS, LRTIs in the first year of life, parental asthma and atopic dermatitis was evaluated. The strength of the association differed with LRTIs in the first year of life, sex, parental education, ETS, parental asthma and atopic dermatitis but only for 'LRTIs in the first year of life' the estimation of the regression coefficient of the interaction term was precise enough (p-value of the interaction term below the α-level of 0.20) to consider modification. This suggests modification of the association between first parent-reported asthma occurrence and systemic antibiotic use in the first year of life by LRTIs in the first year of life. Current IDR comparing (first) asthma occurrence in children who received four or more courses of systemic antibiotics in the first year of life with (first) asthma occurrence in children who received less than four courses revealed a more pronounced (and statistically significant) effect in children with reported LRTIs in the first year of life compared to children with no reported LRTIs in the first year of life (IDR [95% CI]: 8.19 [1.38, 48.75], p = 0.04 versus 1.44 [0.42, 4.93], p = 0.55).

Adjusting for confounding by sex, parental education, day-care attendance and ETS, resulted in a more pronounced association (IDR [95% CI]: 2.52 [0.95, 6.63], p = 0.06). Although our estimation was rather precise, this association is not statistically significant at the predominantly used α-level of 0.05. Other characteristics (age, breastfeeding for at least 6 months and paracetamol (acetaminophen) use in the first year of life) hardly confounded the association, with no changes in the regression coefficient more pronounced than 0.1 decimals. In the final model in the CCA, both sex, parental education, day-care attendance and ETS confounded the association, whereas in the statistical modelling approach with the imputed datasets only parental education and ETS were identified as confounders of the association.

Supplementary table 1: *Results based on crude and adjusted models in the complete case approach for the association between first asthma occurrence in children and excessive systemic antibiotic use (≥4 courses) in the first year of life*

	B	SE	IDR	95% CI	p
Crude model					
Excess systemic antibiotic use in the first year of life [events: n = 47; population moments: n = 147]	0.59	0.38	1.80	(0.85, 3.81)	0.12
Excess systemic antibiotic use in the first year of life (CCA) [events: n = 30; population moments: n = 107]	0.80	0.45	2.24	(0.93, 5.38)	0.07
Adjusted model[a]					
Excess systemic antibiotic use in the first year of life	0.92	0.49	2.52	(0.95, 6.63)	0.06
Evaluation of effect modification by sex					
Crude model					
Excess systemic antibiotic use for sex = Male	0.47	0.56	1.59	(0.53, 4.81)	0.41
Excess systemic antibiotic use for sex = Female	1.28	0.75	3.59	(0.83, 15.54)	0.09
Interaction term	0.81	0.94[†]	-	-	0.38
Adjusted model[b]					
Excess systemic antibiotic use for sex = Male	0.64	0.59	1.90	(0.59, 6.09)	0.28
Excess systemic antibiotic use for sex = Female	1.49	0.82	4.43	(0.89, 22.10)	0.07
Interaction term	0.85	0.98[†]	-	-	0.39
Evaluation of effect modification by parental education					
Crude model					
Excess systemic antibiotic use for parental education = Low	0.47	1.15	1.60	(0.17, 15.27)	0.68
Excess systemic antibiotic use for parental education = High	0.87	0.49	2.40	(0.91, 6.29)	0.07
Interaction term	0.40	1.25[†]	-	-	0.75
Adjusted model[c]					
Excess systemic antibiotic use for parental education = Low	1.26	1.32	3.52	(0.27, 46.44)	0.34
Excess systemic antibiotic use for parental education = High	0.88	0.52	2.40	(0.86, 6.69)	0.09
Interaction term	-0.38	1.38[†]	-	-	0.78
Evaluation of effect modification by ETS					
Crude model					
Excess systemic antibiotic use for ETS = No	0.74	0.48	2.09	(0.81, 5.39)	0.13
Excess systemic antibiotic use for ETS = Yes	1.54	1.4	4.67	(0.30, 73.38)	0.27
Interaction term	0.80	1.49[†]	-	-	0.59
Adjusted model[d]					
Excess systemic antibiotic use for ETS = No	0.81	0.53	2.24	(0.80, 6.27)	0.12
Excess systemic antibiotic use for ETS = Yes	1.84	1.45	6.29	(0.37, 107.61)	0.20
Interaction term	1.03	1.52[†]	-	-	0.50

Evaluation of effect modification by LRTIs in the first year of life					
Crude model					
Excess systemic antibiotic use for LRTIs first year of life = No	0.36	0.59	1.43	(0.45, 4.55)	0.55
Excess systemic antibiotic use for LRTIs first year of life = Yes	1.66	0.82	5.25*	(1.05, 26.20)	0.04
Interaction term	1.30	1.01**	-	-	0.20
Adjusted model[e]					
Excess systemic antibiotic use for LRTIs first year of life = No	0.37	0.63	1.44	(0.42, 4.93)	0.56
Excess systemic antibiotic use for LRTIs first year of life = Yes	2.10	0.91	8.19*	(1.38, 48.75)	0.02
Interaction term	1.74	1.07**	-	-	0.11
Evaluation of effect modification by parental asthma					
Crude model					
Excessive systemic antibiotic use for parental asthma = No	0.94	0.49	2.56	(0.98, 6.68)	0.05
Excessive systemic antibiotic use for parental asthma = Yes	0.92	1.49	2.50	(0.13, 46.77)	0.54
Interaction term	-0.02	1.57†	-	-	0.99
Adjusted model[f]					
Excessive systemic antibiotic use for parental asthma = No	1.07	0.54	2.93*	(1.03, 8.38)	0.04
Excessive systemic antibiotic use for parental asthma = Yes	1.54	1.59	4.64	(0.21, 104.48)	0.33
Interaction term	0.46	1.65†	-	-	0.78
Evaluation of effect modification by atopic dermatitis					
Crude model					
Excessive systemic antibiotic use for atopic dermatitis = No	1.09	0.69	2.99	(0.77, 11.50)	0.11
Excessive systemic antibiotic use for atopic dermatitis = Yes	0.66	0.61	1.94	(0.59, 6.36)	0.27
Interaction term	-0.43	0.92†	-	-	0.64
Adjusted model[g]					
Excessive systemic antibiotic use for atopic dermatitis = No	1.17	0.71	3.24	(0.81, 12.97)	0.10
Excessive systemic antibiotic use for atopic dermatitis = Yes	0.79	0.67	2.20	(0.59, 8.24)	0.24
Interaction term	-0.39	0.94†	-	-	0.68

ETS: Environmental tobacco smoke; LRTIs: Lower respiratory tract infections; β: regression coefficient; SE: standard error; IDR: Incidence density ratio; CI: confidence interval; [a]Adjusted for confounding by sex, parental education, day-care attendance and ETS; [b]Adjusted for confounding by parental education, day-care attendance and ETS and taking into account effect modification by sex; [c]Adjusted for confounding by sex, day-care attendance and ETS and taking into account effect modification by parental education; [d]Adjusted for confounding by sex, parental education and day-care attendance and taking into account effect modification by ETS; [e]Adjusted for confounding by sex, parental education, day-care attendance and ETS and taking into account effect modification by LRTIs in the first year of life; [f]Adjusted for confounding by sex, parental education, day-care attendance and ETS and taking into account effect modification by parental asthma; [g] Adjusted for confounding by sex, parental education, day-care attendance and ETS and taking into account effect modification by atopic dermatitis; *p<0.05; **p for interaction term <0.20; †p for interaction term >0.20

10. Acknowledgments

I still remember the day I received the phone call from my colleague Sofie, telling me that I was selected for the position as an assistant at the University. At the same time I also got an offer from a private company, but after talking with my husband (back then we only knew each other for one year), he thought the assistant position would be perfect for me. And thank you Mohamed, apparently you already knew me very well back then, because today I am happy and grateful for the trajectory I went through.

After approximately three years of mainly being a teaching (and research) assistant, I decided to start pursuing a PhD in epidemiology. Even though it has not always been easy finding my way in this position as an assistant, especially not when I decided to also pursue the Master's degree in Epidemiology on top of everything, I am at peace knowing that I gained many valuable skills.

The journey would not have been possible without my mentor, Joost Weyler. Thank you Joost, for making this journey a wonderful and (luckily) atypical experience. I will always remember your words: "Hayat, this is a PhD, which stands for a philosophy degree. So if you just want to produce four papers and put them together in a book, then I am not the right promotor for you." And even though it took me some time to realize what you meant, I can say now that I would not want the journey to be any different.

Many thanks also to Lidia, my second mentor, for your support, advice and feedback. Thank you for 'adopting' me when you started at the university and Joost was about to retire. Lucky me, to have a supervisor who I can call whenever I am in distress (day or evening) and lucky me to have someone with your expertise close to learn from. I really hope that in the future I will have the opportunity to learn even more from you.

Also many thanks to my other colleagues, without you this journey would not have been bearable. Not that I did not like the job or the work, on the contrary, I love it so much that it hurts when things are not going as you want them to go. You were there for the mental support. They say 'it takes a village to raise a child', well I think we can also say: 'it takes a village to finish a PhD'. Sanne, Charlotte, Katrien, David,… and many others (you know who you are), you were my village! By now we can say that we are not only colleagues, but also friends. Also many thanks to my other friends and family, especially the ones who were cheering for me along the way.

Someone who deserves his own paragraph in this acknowledgement (and even more of course) is my husband, my life companion. Without you, this journey would not have

been possible. You always put yourself aside for me and the children and you always say: “ I want my wife and children to shine in life and I want to facilitate that as much as possible with all my heart”. I do believe that you also deserve your moment of shine, so thank you for what you do for us.

To my two beautiful children, Alina and Elias, I am grateful to be your mom. You made me see life through a whole other perspective. You are way too little to realize what I am actually doing here (except for mama being a scientist which is cool), but I really hope that, when you are older, you will understand the meaning of standing here today. This goes beyond getting a degree. This is also about fulfilling the hope my grandparents and parents (and all first generation migrant workers) had when they decided to move to another country. The hope for a better life. I would not be here if my grandparents and parents were not brave enough to undertake the journey they made. I dedicate this journey to them and I thank them for everything they have done for us. Alhamdoelilah.

11. Curriculum vitae

Personalia

Surname	Bentouhami
Name	Hayat
Date of Birth	01 November 1988
Place of Birth	Mechelen, Belgium
Nationality	Belgian

Degrees

2021	Master of Science in Epidemiology, University of Antwerp *Magna cum laude*
2014	Master of Science in Biomedical Sciences - Clinical research and management and entrepreneurship, University of Antwerp *Cum laude*
2013	Bachelor of Science in Biomedical Sciences, University of Antwerp
2007	Science-mathematics, K.A. Ekeren

Professional experience

2017-present	PhD student in Medical Sciences - Epidemiology, University of Antwerp *Theoretical (object) design in non-interventional causal epidemiological research, a critical appraisal. Issues in studies on the causal role of perinatal factors and the occurrence of asthma in children.*
2021-present	Researcher, epidemiologist, Social Epidemiology and Health Policy, University of Antwerp
2014-2021	Research and Teaching assistant, Epidemiology and Social medicine, University of Antwerp

Academic Training

2021	Alpine Exposome Summer School, Inserm, France
2016	Causal Diagrams for Epidemiological Research, Universitat Pompeu Fabra, Barcelona, Spain
2015-2016	Semester training assistants, ECHO University of Antwerp, Belgium
2014	Residential Summer Course in Epidemiology, EEPE, Firenze, Italy

Scientific publications

Markevych I, Zhao TY, Fuertes E, et al. Residential greenspace and lung function decline over 20 years in a prospective cohort: the ECRHS study. Environment International 2023.

Bentouhami H, Bungwa MK, Casas L, et al. Asthma occurrence in children and early life systemic antibiotic use: an incidence-density study. Allergy, Asthma & Clinical Immunology 2023.

Bentouhami H, Weyler J. Knowledge, perceptions and reporting practices of theoretical design in causal observational epidemiological studies on the role of antibiotic use in the occurrence of asthma in children. Clinical Epidemiology 2023.

Bentouhami H, Casas L, Weyler J. The association between the occurrence of asthma and antecedents of exposure to environmental tobacco smoke in the previous year in children: an incidence-density study. International Journal of Environmental Research and Public Health 2022.

Bentouhami H, Casas L, Weyler J. Reporting of "theoretical design" in explanatory research: A critical appraisal of research on early life exposure to antibiotics and the occurrence of asthma. Clinical Epidemiology 2021.

Van Dyck L, Bentouhami H, Koch K, et al. Exposure to indoor ferromagnetic particulate matter monitored by strawberry plants and the occurrence of acute respiratory events in adults. International Journal of Environmental Research and Public Health 2019.

Lytras T, Beckmeyer-Borowko A, Kogevinas M, et al. Cumulative exposures and lung-function decline in two large general population cohorts. Proceedings of the American Thoracic Society 2021.

Lytras T, Kogevinas M, Kromhout H, et al. Occupational exposures and incidence of chronic bronchitis and related symptoms over two decades: the European Community Respiratory Health Survey. Occupational and Environmental Medicine 2019.

Lytras T, Kogevinas M, Kromhout H, et al. Occupational exposures and 20-year incidence of COPD: the European Community Respiratory Health Survey. Thorax 2018.

Lonnebotn M, Svanes C, Igland J et al. Body silhouettes as a tool to reflect obesity in the past. PloS ONE 2018.

Posters and presentations at scientific conferences

2019	WEON (oral presentation): Use and reporting of theoretical design in epidemiological research: A critical appraisal of research on early life exposures and the occurrence of asthma. Bentouhami H, Weyler J.
2017	ERS (poster discussion): Sex- and age-specific wheeze pattern in the first year of life. Bentouhami H, Weyler J, Van den Eynde K, Oostveen E.
2017	WEON (oral presentation): The impact of aspects of methods design on study results: the case of the nested case-control study. Bentouhami H, Weyler J.
2016	ERS (oral presentation): Nonuniform growth in pediatric lung function between 4 and 14 years of age. Oostveen E, Bentouhami H, Hagendorens M, et al.
2016	WEON (poster): Is exposure to indoor PM, measured by biomagnetic monitoring of strawberry leaves, associated with acute respiratory events in adults? Van Dyck L, Bentouhami H, Koch K, et al.

2015 WEON (poster): Development of a risk score for the prediction of COPD in the European Community Respiratory Health Survey (ECRHS) III cohort (Antwerp participants). Acke S, Atanga M, Bentouhami H, et al.

Membership boards and societies

2020-2021 Member of the Junior Epidemiologists Working Group of the Netherlands Society for Epidemiology

2018-2021 Chair of the communications and student recruitment working group for the epidemiology master's program

2017-2021 Master's epidemiology representative in the Communication and Student Recruitment Committee of the Faculty of Medicine and Health Sciences

2015-2021 AAP representative for the Faculty of Medicine and Health Sciences in the Education Council of the University of Antwerp

2015-2018 AAP representative departmental council of Epidemiology and Social Medicine

2015-2017 AAP representative Faculty Council Medicine and Health Sciences

2014-2017 Member of the education committee of Master of Epidemiology