SUMMARY

The primary aim of this thesis was to collect information and to provide new evidence for monitoring and counselling of women of childbearing age who have been or are exposed to acitretin. The studies in the present thesis were all initiated after the alert on the teratogenic dermatological agent acitretin in October 1990, when it was notified that unexpectedly, the lipophilic compound etretinate was found in plasma of acitretin users. This resulted in an extension of the recommended post-therapy contraception period from 2 months to 2 years, which inevitably had a large impact on acitretin exposed women of childbearing age. In this thesis the evaluation of communication processes, the frequency of the presence of etretinate, the identification of risk factors associated with the presence of acitretin into etretinate, and the toxicologic and teratogenic risks of acitretin are discussed.

In **chapter 2**, the position of the postmarketing surveillance study on acitretin, which is an ad-hoc field-study, is described with respect to the currently available systems, sources and methods for postmarketing surveillance. Field-studies appear to be old-fashioned, and have many disadvantages such as time, cost and validity. However, due to their potential to study rare outcomes or exposure, and their flexibility in measuring different outcomes, which often cannot be measured in automated databases, such as plasma levels of specific drug, quality of life or benefits of drugs, ad-hoc field-studies still deserve a place in modern pharmaco-epidemiology. In addition to the different systems, novel methods in (pharmaco)-epidemiology such as the case-crossover, nested case-control and case-cohort designs are briefly described. In this thesis the follow-up, case control and case-crossover design were applied.

In **chapter 3** the preclinical, clinical, and postmarketing experience with acitretin therapy are delineated. The historical development of retinoids was based on the toxicity/efficacy ratio and evolved from vitamin A to the first (isotretinoin), second (etretinate, acitretin) and to the third generation retinoids (motretinide). The molecular mechanism of action is not fully elucidated yet. Acitretin is merely used for the treatment of keratinization disorders such as psoriasis, ichthyosis, Darier's disease etc. Acitretin is a lipophilic compound which is extensively bound to plasma proteins (>99%). Oral absorption is variable and dependent on food, acitretin does not accumulate in fat and has an elimination half-life of about 50 h. It is metabolised by isomerization to the cis-isomer, oxidation and finally glucuronidation. Apparently, acitretin can be esterified to its ethanol-ester etretinate. The toxicity resembles the pattern of effects observed in the hypervitaminosis A syndrome and include, mucocutaneous, hepato, visual, and central nervous toxicity and is often a reason to quit therapy. The most important disadvantage of acitretin is its teratogenic potency, which is the motive for the majority of studies in this thesis. Most of the problems concerning the teratogenic potency are related to uncertainties: the duration of the period of excess teratogenic risk after discontinuation of acitretin, and the teratogenic threshold in plasma of acitretin and etretinate are still unknown.

In **chapter 4**, we discuss how an ad-hoc cohort of acitretin-exposed women of childbearing age was recruited following the alert on acitretin. Recruitment occurred by dermatologists, and pharmacists plus dispensing general practitioners (GPs). We described the velocity of, and response to the recruitment procedures, and the representativeness of the recruited cohort. It was also studied whether the individuals who gave informed consent would have preferred to be recruited by either dermatologists or pharmacists, and whether the information obtained from pharmacists and dispensing GPs was valid. The results of this study show that pharmacists and dispensing GPs (drug dispensers) recruited fast, with none or little selection and attained 42% response. Dermatologists recruited slow, selective and with 24% response. The majority of women (60%) recruited by dermatologists would have given informed consent if they were recruited by their pharmacist, as likely.

In **chapter 5**, the communication procedures and effects were evaluated which followed the alert on acitretin. The penetration of direct mail from the health authorities and from the pharmaceutical company ranged from 97-98% and 65-94% among health professionals. The population at risk was informed via personal communication with health professionals, and/or the mass media. Of the women at risk, 19% were contacted by the dermatologist, 30% by the GP, and 39% by the pharmacist, 35% was never informed by any health professional. The results of this study show that the Dutch health care system is adequately equipped for effective communication between health authorities, pharmaceutical industry and health professionals. Due to problems with identification according to past exposure, subsequent personal communication between health professionals and the population at risk was inadequate. This study shows that the role of pharmacists in personal communication should be increased, as they can rapidly identify persons at risk as a result of previous exposure to specific drugs.

In **chapter 6**, an overview is given of the different methods to measure and define drug exposure. Throughout this thesis we used the legend duration method to define exposure to acitretin. Sources for collection of data on drug intake were interview data, medical records and plasma levels. The reliability of the methods was good as was calculated in appendix B.

In **chapter 7**, utilization characteristics of acitretin were described. In addition it was investigated whether those utilization characteristics changed after the alert on the extended contraception period. The results showed that women who suffered from severe non-psoriatic keratinization disorders were exposed to acitretin more (longer) during the study period. Also they were more likely to have used etretinate in the past, were more compliant, but they were not prescribed higher daily dosages than women suffering from psoriasis.

In **chapter 8**, we estimated the frequency of plasma etretinate concentrations in women of childbearing age who were treated with acitretin. A cross-sectional study, and a one-year follow-up study were conducted in 181 and 62 women, respectively. Two analytical assays, with different limits of detection were used to measure plasma etretinate and acitretin concentrations. The initial, commonly used assay (limit of detection: 1 ng/ml) yielded highly variable point prevalences (range:15-46%) among us-

ers of acitretin, and a small cumulative incidence of etretinate in plasma (30%, $CI_{95\%}$ 12%,47%). The second assay (limit of detection of 0.1ng/ml), resulted in more consistent, reproducible point prevalences (range: 64-77%), at the different sampling times. The 9-months cumulative incidence was 78% ($CI_{95\%}$:60%, 96%). Among stoppers of acitretin therapy the drug was detectable in plasma up to 30 months after discontinuance. This study showed that nearly all female users of acitretin have significant etretinate plasma concentrations, and etretinate or its metabolite are detected in plasma much longer than the expected maximum of 2 years.

In **chapter 9**, the predictive value of plasma etretinate measurements in comparison with the presence of etretinate in fat was measured. The prevalences of detectable etretinate concentrations were 45% and 83% in plasma and subcutaneous tissue, respectively, among current acitretin users and 18% and 86% among those who had stopped acitretin therapy. Inability to detect plasma etretinate is a poor predictor for the absence of etretinate in fat. Acitretin and/or etretinate were detectable in fat and in some cases in plasma from women who had ceased acitretin therapy for up to 29 months.

In **chapter 10**, we describe the implementation of the analytical method to determine etretinate and acitretin simultaneously in plasma, fat and liver tissue of rats. This analysis was set up to conduct an experiment with the aim to investigate the effects of fasting, or food deprivation, on etretinate and acitretin plasma concentrations of rats which were previously loaded with etretinate. Knowledge of the effect of fasting on the disposition of etretinate might be useful for counselling of women with etretinate subcutis concentrations who want to become pregnant. This pilot experiment showed that fasting or loss of weight after etretinate treatment may quickly raise acitretin (metabolite) plasma levels. Upon extrapolation to humans, these findings could contribute to monitoring of women, since a short period of fasting might yield detectable plasma levels.

In **chapter 11**, we describe the study which aimed to identify risk factors which are associated with the presence of etretinate in plasma of acitretin users. Risk factors were sought in factors which might influence absorption, distribution and metabolism of acitretin and included diet, demographics, medical history, drug and alcohol intake, smoking, abnormal blood chemistry, prior etretinate treatment, and recency of acitretin intake. Out of the 181 females, 52 (29%) were currently exposed to acitretin. In the total of 181, 23 (13%) women had etretinate levels above 1 ng/ml. Variables which were independent predictors for the presence of etretinate in plasma included current exposure to acitretin (OR=43, $CI_{95\%}$: 9.5, 387); high accumulated intake of acitretin (OR=26, $CI_{95\%}$ 5.0, 247); more than 2 glasses of alcohol per day (OR=10 $CI_{95\%}$ 1.2, 121), past exposure to etretinate (OR=8.8 $CI_{95\%}$ 2.9, 32); Quetelet indices above 30 (kg/m^2) (OR=5.5 $CI_{95\%}$ 1.01, 25), suffering of heartburn (OR=4.2 $CI_{95\%}$ 1.5, 12), intake of acitretin in the evening (OR=4.5, $CI_{95\%}$ 1.7, 38), concomitant intake of highly protein-bound drugs (OR=3.9, $CI_{95\%}$: 1.1-13), more than 5 prescriptions of Cyt P-450 inducing drugs concomitant with acitretin (OR= 3.5, $CI_{95\%}$:1.0-14), and abnormal low serum protein levels (OR:3.9 $CI_{95\%}$: 1.1-13). High intake of mono-disaccharides was negatively associated (OR=0.2 $CI_{95\%}$ 0.04, 0.9). In the multivariate analysis,

the high intake of saturated fat, singular unsaturated fat, and animal protein was associated with the presence of etretinate in plasma (OR_{adj}=17, $CI_{95\%}$ 1.7, 18), (OR_{adj}=10.7, 1.7) and (OR_{adj}=7.7, $CI_{95\%}$ 1.1, 56), respectively.

In **chapter 12**, a 20-week-old male fetus is described with multiple congenital anomalies who was exposed to acitretin in the first trimester of pregnancy. The fetus showed severe symmetric anomalies of upper and lower limbs, mandibulofacial dysostosis like craniofacial anomalies, ear anomalies and an ASD. Although the craniofacial anomalies resemble the malformation observed in the classic 'retinoic acid embryopathy', limb malformations were seldomly reported after maternal use of vitamin A congeners. This case emphasises again that extreme care and precaution are needed before prescribing a potentially teratogenic drug to a fertile woman.

In **chapter 13**, we describe the methodology of follow-up and investigation of pregnancy outcomes in a fixed population-based acitretin exposure cohort in the Netherlands. Children conceived within and after the 2-year post-therapy contraception, who originated from the prospective cohort were examined for major and minor malformations at the age of 1.5-2 years. In total 28 pregnancies occurred in the cohort, 16 within, and 12 after 2 years post-therapy. Of the women at risk to become pregnant 10% conceived within two years. Seven women (5%) conceived despite the use of contraceptives, 6 of them had used low dose oestrogen pills. In total 6 pregnancies ended in a spontaneous, and 4 in an elective abortion. The percentage of pregnancies ending in spontaneous abortions did not substantially differ in the series conceived within and after 2 years, respectively. A total of 18 liveborn babies are potentially available for dysmorphologic examination. The six children examined yet, all showed minor malformations, whereas 3 showed more serious medical problems. Although we could not make inferences on the malformations associated with the previous use of acitretin yet, this study showed that too many women got pregnant within the recommended two year contraception period. Hence. adequate contraception and potential threats to its efficacy need to be surveilled closely by physicians and pharmacists.

In **chapter 14**, we described the dysmorphology and neurobehavorial characteristics of a 9-year old boy whose mother conceived 10 months after cessation of etretinate therapy. The boy shows a few minor craniofacial malformations which have been associated with the retinoic acid embryopathy. In addition his intelligence is far below normal (IQ: 68) and difficulties exist in those neurologic functions, which also seem to be affected most often after isotretinoin exposure. This report shows that potentially etretinate, and acitretin as its teratogenic metabolite may cause neurobehavioral problems.

In **chapter 15**, we aimed to make a rough inventory of the known and unknown side effects of acitretin experienced by women of childbearing age. Spontaneously reported, but hitherto unknown effects included changes of pigmentation, eczema, change of temper/ mood/ concentration, vertigo, edema, arrhythmia, vulvo-vaginal infection, and more serious blood loss during menstruation. The crude cumulative incidence of systematically asked known adverse effects corresponded with the reported rates in trials except for ocular dryness/conjunctivitis, diaphoresis and loss of

hair which were all reported more frequently. Among the systematically asked hitherto unknown adverse effects, vulvo-vaginal infection (21%), and paraesthesia (18%) were reported most often by our study population. Vertigo was reported by 11% of the women. Vertigo and vulvo-vaginal infections were perceived as most severe, whereas only the latter condition was largely treated with medications.

In **chapter 16**, we estimated the risk of vulvo-vaginal candidiasis among the users of acitretin through prescription sequence analysis. The positive predictive value of the proxy drug for vulvo-vaginal candidiasis ranged between 57-100%, the sensitivity was 87% and the specificity was 99%. The crude incidence rate ratio for vulvo-vaginal candidiasis following acitretin exposure was 2.8($CI_{95\%}$:1.1-7.1). The pooled Mantel-Haenszel incidence rate ratio was 3.3($CI_{95\%}$:1.1-9.6) after stratification for accumulated level of exposure. Patient-stratified analysis on the subgroup of cases (n=15) revealed an odds ratio of 6.5 ($CI_{95\%}$:2.3-18.2).

In **chapter 17**, a follow-up study is presented which describes the association between acitretin and the occurrence of medically treated vertigo. Although not significantly, the relative risk of vertigo was increased during acitretin exposure (6.1, $CI_{95\%}$: 0.78-50.8). Vertigo was perceived as very serious by the patients, therefore additional studies should be done with a larger sample size as to further explore this suggested association.

In **chapter 18**, we intended to measure the perception of teratogenic risk of acitretin by the women, physicians and regulatory authorities in three situations, which differed as regards time of discontinuance of therapy and plasma acitretin/etretinate levels. The results showed that there is an unjustified reliance on the safety of the 2 year criterium. Conception whilst acitretin was detectable in plasma was perceived most risky, most respondents perceived the risk as much to extremely higher compared to the average risk. The risk of malformations upon conception within 2 years was considered higher than that after two years. The risk perception of the regulatory authority was fully in line with the womens' and physicians' perceptions. Communication about their own plasma levels did not change the risk perception of women, however, half of the women wanted additional information.

Several studies in this thesis have shown that personal communication with the patients at risk is not always up to standard, mostly since it is difficult for physicians to identify users of a specific drug. In addition it was shown throughout this thesis that the frequency of the presence of etretinate in plasma and fat is high, that acitretin can be converted into etretinate in the body and that the period of excess risk can be longer than 2 years. It was shown that plasma measurements are not capable of predicting the absence of etretinate in fatty tissue very well, and that the frequency of etretinate as well as the predictive value depend highly on the analytical method being applied. Etretinate might be mobilized due to loss of weight, and potentially other factors. We observed that acitretin very likely is a human teratogen and may also cause neurobehavioral problems. Following our results, women of childbearing age should not be prescribed acitretin. The women who already use acitretin and want to conceive should refrain from alcohol and other identified risk factors for the presence of etretinate, they should be told that the period of excess risk may be longer than 2

years, as to prevent unjustified reliance. They should be offered monitoring in plasma and subsequently fat tissue by means of the most sensitive analytical assay, with a limit of detection of 0.1 ng/ml preferably.