What is considered cardiotoxicity of anthracyclines in animal studies

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Abstract. Anthracyclines are commonly used anticancer drugs with well-known and extensively studied cardiotoxic effects in humans. In the clinical setting guidelines for assessing cardiotoxicity are well-established with important therapeutic implications. Cardiotoxicity in terms of impairment of cardiac function is largely diagnosed by echocardiography and based on objective metrics of cardiac function. Until this day, cardiotoxicity is not an endpoint in the current general toxicology and safety pharmacology preclinical studies, although other classes of drugs apart from anthracyclines, along with everyday chemicals have been shown to manifest cardiotoxic properties. Also, in the relevant literature there are not well-established objective criteria or reference values in order to uniformly characterize cardiotoxic adverse effects in animal models. This in depth review focuses on the evaluation of two important echocardiographic indices, namely ejection fraction and fractional shortening, in the literature concerning anthracycline administration to rats as the reference laboratory animal model. The analysis of the gathered data gives promising results and solid prospects for both, defining anthracycline cardiotoxicity objective values and delineating the guidelines for assessing cardiotoxicity as a separate hazard class in animal preclinical studies for regulatory purposes.

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Abbreviations: LV, left ventricular; LVEF, LV ejection fraction; LVFS, LV fractional shortening; BNP, brain natriuretic peptide; PWT, posterior wall thickness; AWT, anterior wall thickness; SWT, septal wall thickness; BP, blood pressure; HR, heart rate; LVSP, LV systolic pressure; LVDP, LV diastolic pressure; LVEDd, LV end-diastolic diameter; LVESd, LV end-systolic diameter; LVEDV: LV end-diastolic volume; LVIDd, LV internal diastolic diameter; LVISd, LV internal systolic diameter; LVPWs, LV systolic wall thickness; LVPWd, LV diastolic wall thickness; IVSd, intraventricular septum in diastole; LAD, left atrial diameter; AOD, aortic diameter; ACEIs, angiotensin converting enzyme inhibitors; ARBs, angiotensin II receptor blockers

Key words: anthracyclines, echocardiography, ejection fraction, fractional shortening, rats

Introduction

Chemotherapeutics cardiotoxicity is a major concern for clinicians treating different kinds of cancer, as it seriously affects their treatment options and the survival of the patient. The cut-off values for the identification of cardiotoxicity caused by chemotherapeutics in humans differ between the American and European guidelines: the definition considers a lower cut-off value of normality for the left ventricular ejection fraction (LVEF) of 50% in Europe (1) and 53% in the USA (2). Both Guidelines emphasize that a drop of LVEF compared to the patient's previous values is also required. This definition is crucial for patients and clinicians, as patients presenting this decline in cardio-imaging indices of cardiac function should be treated with angiotensin converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs) in

combination with β-blockers (3); nevertheless, modifications of anticancer treatment in such patients remain a matter of discussion among different specialists.

In animal studies, where new anticancer substances are evaluated and different agents are tested to overcome anticancer drugs cardiotoxicity, identification of the extent of cardiotoxicity is crucial and necessary for the evaluation of any favourable effects of the counteracting agent (4). In this regard, cardiac imaging is more often used at analogy to the clinical setting. Biomarkers and clinical signs of heart failure are also taken into consideration, but cardiac imaging in animal studies has gained momentum.

Anthracyclines are a class of drugs used in cancer chemotherapy isolated from *Streptomyces bacterium*. These compounds are used to treat many cancers, including leukemias, lymphomas, as well as breast, stomach, uterine, ovarian, bladder cancer, and lung cancers (5-7). The first anthracycline discovered was daunorubicin (trade name Daunomycin), which is produced naturally by *Streptomyces peucetius*, a species of actinobacteria. Clinically, the most important anthracyclines are doxorubicin, daunorubicin, epirubicin and idarubicin. Anthracyclines, which are considered as well-established cardiotoxic compounds causing myocardial suppression in a considerable number of patients, are also used in animal studies as an easy and low-cost method to introduce a model of dilated cardiomyopathy (8), as opposed to interventional research animal models of infarction and myocardial ischaemia [e.g., permanent ligation of the left anterior descending artery (LAD) or cryo-pen application on the surface of the heart leading to cryo-scar ischemia]. Different animal species and various anthracyclines dosing and administration schemes have been applied in the literature for the development of anthracyclines cardiotoxicity (9) and monitoring of the progress thereof, as well as testing different compounds/schemes for ameliorating myocardial damage. To monitor cardiotoxicity caused by anthracyclines, cardiac imaging is primarily used and secondarily, biochemical markers.

At the same time, other pharmaceutical compounds, such as anabolic steroids, along with everyday chemicals, such as metals and pesticides, have been implicated to adversely affect cardiac pathology causing function impairment (10). Toxicity and risk for human health posed by chemicals are well controlled at a European level through a thoroughly developed regulatory network. Nevertheless, cardiotoxicity is not described as a separate hazard class and no specific classification criteria are available in order to legally classify chemicals well in advance as cardiotoxic and avoid potential long-term cardiovascular complications, which could significantly burden any national health system.

But, what is considered cardiotoxicity of anticancer agents and specifically anthracyclines when parameters of cardiac imaging are monitored in animal studies? Is there a uniformity in animal models of anthracyclines cardiotoxicity induction and most importantly, do all studies describe the same decline of myocardial function? Addressing these issues could be of wider use both in clinical medicine and practice, when assessing agents employed for salvation to cardiotoxic complications during oncology treatment, for example, as well as to regulators, when trying to establish reference values in echocardiographic function representing cardiotoxicity induced in animals by chemicals.

In the current in depth review, the identification of most commonly used metrics of myocardial function in animal studies of anthracycline induced cardiotoxicity are presented, along with the range of these values differentiating normal cardiac function from animals with pathological echocardiographic findings indicative of anthracycline cardiotoxicity as per author presentation.

Materials and methods

PubMed electronic database was systematically searched to detect all original research studies published until March 1, 2020, according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement (11). The specific literature search strategy used was: [AND (“*rats*” OR “*doxorubicin* OR “*echocardiography*” OR “anthracycline” OR “*ejection fraction*”)] either in the Title, or the Abstracts. The reference list of the retrieved studies was further evaluated for the relevance of the subject and the eligibility by screening the titles/abstracts of full papers. The non-English citations (<5) were reviewed separately. Animal data only from rat species were assessed, as it is evident from the search string. All types of citations other than original research studies (e.g., review articles) were excluded. Two authors (NG and CT) independently assessed the title and the abstract content (or both) of each record retrieved to decide which studies should be further evaluated and extracted all data. Disagreements were resolved through consensus or by consultation with a third author (KT). A final draft of the manuscript was prepared after several revisions and approved by all authors. In total, 86 published manuscripts on animal studies were considered for the systematic review (Fig. 1).

Despite the small size of the rat heart and the fast heart rate, echocardiography is systematically used in the evaluation of rat heart function (12). Data for 2 main indices of LV contractility were extracted from the list of studies.

The first index is LV fractional shortening (FS) and is calculated by the formula: FS (%) = [LV end-diastolic diameter (LVD_d) minus LV end-systolic diameter (LVD_s)]/LVD_d x 100.

LVEF is the second and more common, index of LV contractility. EF can be calculated from the equation: EF (%) = [(LVD_d^3 - LVD_s^3) / LVD_d^3] x 100 (13) or from the equation: EF (%) = (LVEDV-LVESV)/LVEDV x 100, where LVEDV is the LV end-diastolic volume and LVESV is LV end-systolic volume (12).

Results

A summary of the studies reviewed in the present report is presented in Table I.

In Figs. 2-5, the normal and suppressed values of the two main echocardiographic indices discussed, %EF and %FS, respectively, are presented. Reported baseline (normal) %EF values in rats vary (55-96.5%). In 78.2% of the studies reviewed, normal values range from 70 to 90%. High %EF values (>90%) are reported in 14% of the studies. In contrast, normal %FS values present even higher variability (25-84.2%).

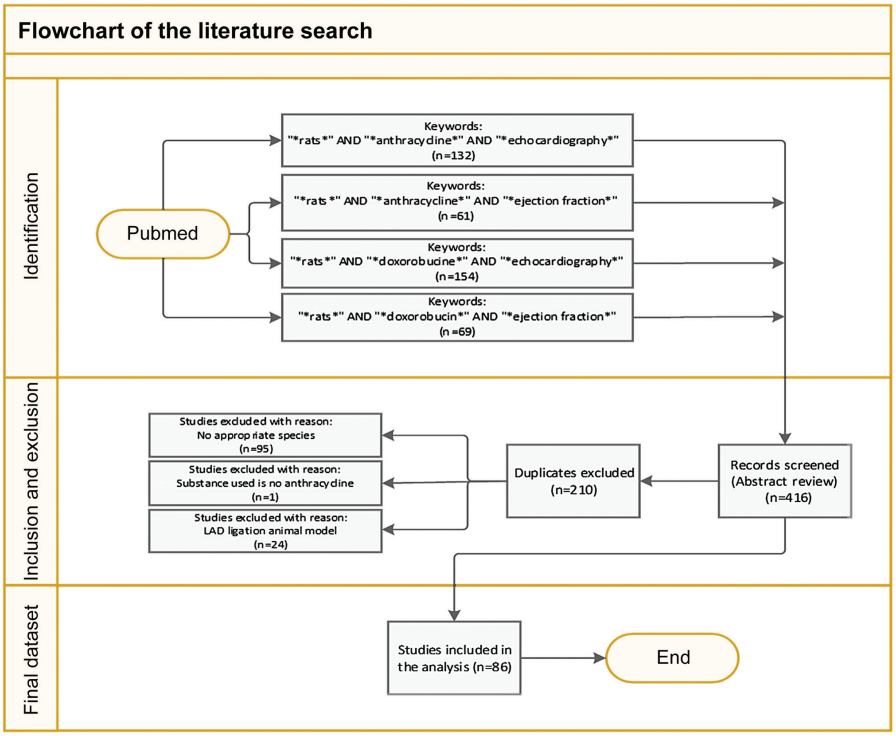

Figure 1. Prisma flow chart (literature search) for the present study design.

The majority (66.7%) of the values, though, are reported to be within the range of 40 and 60%.

Exposure to anthracyclines suppresses both echocardiographic indices. In the 86 studies reviewed in the present report, Doxorubicin is almost universally used to induce cardiotoxicity, along with Daunorubicin and Epirubicin in two studies (Table I). The structures of the three anthracyclines used are presented in Fig. 6. Anthracyclines were administered with order of appearance either via intraperitoneal injection, intravenous injection or orally with the feed. The doses were administered once, twice, three times per week. The duration of the dose administration spans from one week to ten weeks. In most of the experiments, the benchmark for terminating the administration was the proof of cardiac toxicity. The echocardiography values suggest that there is no specific dose regime threshold which indicates the establishment of the effect, but it is specific to each experiment and probably dependent on other factors such as age and general condition of the animals.

The suppressed %EF values reported from rats after anthracyclines administration vary from 31 to 91% (Fig. 4). EF values 50-80% are reported in 72.3% of the studies reviewed. Suppression of the %EF due to anthracycline administration varies from 10 to 40% compared to the normal values in more than two thirds of the studies reviewed (71.7%) (Fig. 7). On the other hand, suppressed %FS values ranging from 14 to 71.8%, present a more narrow distribution (%FS values 20-50% in 84.6% of the studies). As shown in Fig. 7, a more equal distribution of the %FS suppression due to anthracycline toxicity is observed with approximately one fourth of the studies reporting 20-30% and 30-40% suppression, respectively. It is evident from Figs 8 and 9 that normal and suppressed %EF and %FS values separate sufficiently well. The rat strain does not seem to influence either the normal or the suppressed %EF and %FS values (Fig. 10).

Only 11 studies used an acute administration scheme, with 3-20 mg/kg bw anthracycline single injection either intravenously or intraperitoneally. Most of the studies used a prolonged administration period, from 2 weeks (33 studies) up to 10 weeks, and cumulative doses ranging from 1 to 20 mg/kg bw. All dosage schemes were carefully selected to induce cardiotoxicity and did not seem to affect the suppression of %EF and %FS monitored.

Discussion

Myocardial contractility suppression due to anthracycline administration is of increasing interest and represents a major challenge in the clinical setting. At the same time in a preclinical stage it serves as a model for the assessment of both new chemotherapeutic and cardioprotective agents to be introduced in clinical practice. The myocardial toxicity of anthracyclines is known to be affected by sex and age, along with a number of cardiovascular risk factors and comorbidities (99). It is found that anthracycline related congestive heart failure reaches 10% of patients older than 65 years at usual doses (100). While in early studies it was thought that EF cannot accurately predict congestive heart failure attributed to doxorubicin (100), current perspective is that anthracycline-related cardiotoxicity is manifested by a progressive continuous decline in LVEF (1) and identifying subclinical myocardial dysfunction related to anthracycline treatment has great therapeutic implications (2).

Preclinical animal studies are essential in cancer chemotherapy research along with the evaluation of the cardiotoxic propensity of the chemotherapeutic agents. The current recommendations for prevention of cardiac events from cancer chemotherapies are

Table I. Treatment protocol and main findings of the studies that examined anthracyclines cardiotoxicity in rats reviewed in the present report.

Publication	No. of animals/rat strain/sex	Anthracycline administered	Anthracycline total dose	Duration	Summary of findings	Calculations
Zhang *et al* (14)	30/Sprague Dawley rats/male	Doxorubicin (brand name Adriamycin)	1 mg/kg	Daily doses for 2 weeks	Cardiac dysfunction (parameters monitored: diastolic left ventricular internal dimension, systolic left ventricular internal dimension, LVEF and LVFS)	Values calculated manually by the authors of this review
Tian *et al* (15)	70/Sprague Dawley rats/Male	Doxorubicin	3.0 mg/kg	Once a week for 6 weeks	Cardiomyopathy	Values provided in the manuscript
Andreadou *et al* (16)	90/Wistar rats/male	Doxorubicin	18 mg/kg, ip	6 equal doses for 2 weeks	Cardiomyopathy (parameters monitored: cardiac geometry, function and histopathology)	Values provided in the manuscript
Oliveira *et al* (17)	20/Wistar rats/male	Doxorubicin	5 mg/kg, ip	Once a week for 4 weeks	Ventricular dysfunction	Values provided in the manuscript
Hydock *et al* (18)	46/Sprague-Dawley rats/Male	Doxorubicin	10 mg/kg ip	Acute administration (bolus)	Parameters altered: LVFS and LVPWT	Values provided in the manuscript
Fernandez-Fernandez *et al* (19)	36/Sprague-Dawley rats Wistar rats Fischer-344 rats/NM	Doxorubicin	18 mg/kg	Over 12 days	Cardiac function altered (LVFS, left ventricular developed pressure, contractility and relaxation, cardiac capillary permeability)	Values provided in the manuscript
Todorova *et al* (20)	27/Fisher 344 rats/female	Doxorubicin	12 mg/kg (1.5 mg/kg each)	Twice per week for 4 weeks	Parameters monitored: Plasma levels of troponin I Left ventricle (LV) function, LV PWT, LV volume, LVEF, LVFS	Values provided in the manuscript
Vasić *et al* (21)	68/Wistar rats/male	Doxorubicin	15 mg/kg ip	Every other day for 2 weeks	Parameters monitored: Echocardiography, serum cardiac troponins, heart rate variability and blood pressure variability	Values provided in the manuscript
Mathias *et al* 22)	64/Wistar rats/male	Doxorubicin	20 mg/kg ip	Acute administration (a single injection)	Altered LVFS	Values provided in the manuscript
Wang *et al* (23)	40/Sprague-Dawley rats/male	Doxorubicin (brand name Adriamycin)	15 mg/kg ip	Acute administration (a single injection)	Altered LVEF, LVFS and LV outflow	Values calculated manually by the authors of this review

Table I. Continued.

Publication	No. of animals/rat strain/sex	Anthracycline administered	Anthracycline total dose	Duration	Summary of findings	Calculations
Arozal *et al* (24)	25/Sprague-Dawley rats/male	Daunorubicin	3 mg/kg/day (18 mg/kg total dose)	Every other day for 12 days	Altered cardiac function (haemodynamic status and echocardiography)	Values provided in the manuscript
Argun *et al* (25)	40/10-week-old Wistar albino rats/male	Doxorubicin	4 mg/kg/dose to a cumulative dose of 16 mg/kg, ip	Twice a week for 2 weeks	Parameters monitored: Serum BNP and C-type natriuretic peptide LV functions by echocardiography and histological assessment	Values provided in the manuscript
Tatlidede *et al* (26)	32/Wistar albino rats of both sexes	Doxorubicin	20 mg/kg, ip	Every other day for 2 weeks	Parameters monitored: BP and HR, echocardiography Lactate dehydrogenase	Values provided in the manuscript
Razmaraii *et al* (27)	24/adult Wistar rats/male	Doxorubicin	2 mg/kg/48 h	Over a 12-day period	Parameters monitored: LVSP, LVDP, rate of rise/drop of LV pressure, LVEF, LVFS, contractility	Values provided in the manuscript
Gziri *et al* (28)	43/ pregnant Wistar rats/female	Doxorubicin	10 or 20 mg/kg i.v.	On 18th day of pregnancy	Altered left ventricular function	Values provided in the manuscript
Oliveira *et al* (29)	29/adult Wistar rats/male	Doxorubicin	Accumulated doses of 8 (n=8), 12 (n=7), and 16 (n=7) mg/kg, ip	Four weekly injections over 8 weeks	Myocardial fibrosis Altered left ventricular systolic function	Values provided in the manuscript
Carvalho *et al* (30)	64/Wistar rats/male	Doxorubicin	20 mg/kg, ip	Acute administration (a single injection)	LVEF monitored	Values provided in the manuscript
Stewart *et al* (31)	72/Sprague Dawley rats/male	Doxorubicin	15 mg/kg, ip	Acute administration (a bolus injection)	Parameters monitored: LV septal and PWT, LVESd, LVEDd, mitral and aortic valve blood flow profiles, heart dimensions	Values provided in the manuscript
Polegato *et al* (32)	35/Wistar rats/male	Doxorubicin	20 mg/kg, ip	Acute administration (a single dose)	Parameters monitored: LVFS, isovolumetric relaxation time and myocardial passive stiffness	Values provided in the manuscript
Lee *et al* (33)	20/Sprague Dawley rats/male	Doxorubicin	Cumulative dose: 20 mg/kg, ip	Once every two days for 6 times	Impaired LV function and performance	Values calculated manually by the authors of this review

Table I. Continued.

Publication	No. of animals/rat strain/sex	Anthracycline administered	Anthracycline total dose	Duration	Summary of findings	Calculations
Cheah *et al* (34)	29/Wistar rats/male	Doxorubicin	5 mg/kg, iv	Acute administration (a single dose)	Parameters monitored: BP, HR, LVED volume, other echocardiographic parameteres	Values provided in the manuscript
Li *et al* (35)	48/adult Sprague-Dawley rats/male	Doxorubicin	Cumulative dose: 16 mg/kg, ip	Over a 4-week period	Parameters monitored: serum BNP level LVEDd, LVESd, LVEF, LVFS	Values provided in the manuscript
Dundar *et al* (36)	28/adult Wistar albino rats/female	Doxorubicin	15 mg/kg, ip	Acute administration (a single dose)	Parameters monitored: LVIDd and LVISd via the parasternal long axis two-dimensional images. LVFS and LVEF	Values provided in the manuscript
Barış *et al* (37)	31/Sprague-Dawley rats/male	Doxorubicin	25 mg/kg, ip	For 12-14 days	Parameters monitored: left ventricular ejection f raction (LVEF), LVFS and mitral lateral annulus (s') velocity + left ventricular end-diastolic and end-systolic diameters	Values provided in the manuscript
Lu *et al* (38)	60/Sprague-Dawley rats/male	Doxorubicin	2.5mg/kg/week, ip	For 6 weeks	Parameters monitored: LVFS and LVEF	Values provided in the manuscript
O'Connell *et al* (39)	115/adult Wistar rats/male	Doxorubicin	2.5 mg/kg, ip (cumulative dose 15 mg/kg)	6 doses over a period of 2 weeks	Parameters monitored: left ventricular systolic and diastolic dimensions and EF	Values provided in the manuscript
			2 mg/kg, ip (cumulative dose 18 mg/kg)	Once a week for 9 weeks		
Chang *et al* (40)	71/Sprague-Dawley rats/nm	Doxorubicin	3 mg/kg/day, iv	Once a week for 6 weeks	Parameters monitored: SWT and PWT, LVED dimensions, LVES dimensions, LVEF	Values provided in the manuscript
Teng *et al* (41)	46/Sprague-Dawley rats/male	Doxorubicin	2 mg/kg, ip	Once a week for 8 weeks	Parameters monitored: LVED dimensions, LVES dimensions, FS	Values provided in the manuscript
Kim *et al* (42)	61/Sprague-Dawley rats/male	Doxorubicin	1.25 mg/kg, ip	Every other day for 1 month (16 times)	LV systolic/diastolic dysfunction	Values provided in the manuscript
Kondru *et al* (43)	24/Wistar rats/male	Doxorubicin	2 mg/kg, ip	Once in a week for 5 weeks	Myocardial dysfunction	Values calculated manually by the authors of this review

Table I. Continued.

Publication	No. of animals/rat strain/sex	Anthracycline administered	Anthracycline total dose	Duration	Summary of findings	Calculations
Moriyama *et al* (44)	66/Crl:CD(SD) rats/male	Doxorubicin	2 mg/kg, iv	Once weekly, for 6 weeks	Parameters monitored: LVEDd, LVESd, LVFS	Values provided in the manuscript
Burdick *et al* (45)	20/Crl:CD(SD) rats/male	Doxorubicin	2 mg/kg, ip	Once a week for 6 weeks	Parameters monitored: LVFS	Values calculated manually by the authors of this review
Ammar *et al* (46)	50/Wistar rats/male	Doxorubicin	2.5 mg/kg, ip	3 times a week for 2 weeks	Parameters monitored: LVED dimensions and LVSD dimensions, FS	Values calculated manually by the authors of this review
Calvé *et al* (47)	21/Sprague-Dawley rats/female	Doxorubicin	3 mg/kg	Acute administration (on postnatal day 26th)	Parameters monitored: IVSd, LVPWd, LVIDd, LVISd	Values provided in the manuscript
Shen *et al* (48)	150/Sprague-Dawley rat/male	Doxorubicin	1 mg/kg, ip 2 mg/kg, ip (cumulative dose 12 mg/kg)	Twice a week Once a week for 6 weeks	Parameters monitored: LVESd, LVEDd, LVEF	Values provided in the manuscript
Wu *et al* (49)	32/Sprague-Dawley rat/male	Doxorubicin	2.5 mg/kg, ip (cumulative dose 15 mg/kg)	Every second day for 6 times	Parameters monitored: LVEDP, LVESP and left ventricular pressure (±dP/dtmax), LVEF and LVFS	Values calculated manually by the authors of this review
Shoukry *et al* (50)	32/Wister rats/male	Doxorubicin	2.5 mg/kg, ip	2 weeks	Parameters monitored: LVIDd, LVIDs, LVFS and LVEF	Values calculated manually by the authors of this review
Niu *et al* (51)	26/Sprague Dawley rats/male	Doxorubicin	Each dose consisted of 1, 1, 2, 2, 3, 3, 4 and 4 mg/kg, ip (cumulative dose 20 mg/kg)	For 2 weeks on days 1st, 3rd, 5th, 7th, 9th, 11th, 13th and 15th, respectively	Parameters monitored: IVSd, IVSs, LVPWd and LVPWs, LVIDd, LVIDs were measured on left ventricular long-axis areas. LVEF and LVFS	Values provided in the manuscript
Boutagy *et al* (52)	20/Wistar rats (Crl:Wl)/male	Doxorubicin	2.15 mg/kg, ip (cumulative dose 15 mg/kg)	Every 3 days for 21 days	Impaired systolic function and LV volumes and dimensions. Parameters monitored: echocardiographic variables (LVEF, global longitudinal strain, global radial strain, LVEDV, LVESV, relative PWT	Values calculated manually by the authors of this review

Table I. Continued.

Publication	No. of animals/rat strain/sex	Anthracycline administered	Anthracycline total dose	Duration	Summary of findings	Calculations
Lee *et al* (53)	150/Fischer rats/male	Doxorubicin	2.5 mg/kg, ip (cumulative dose 15 mg/kg)	Every other day for 2 weeks	Altered LV function Parameters monitored: LVFS, LVEDd and LVESd, LV end diastolic volume (LVEDV), right basal ventricular diastolic diameter (RVD1), and the RV fractional area change (RVFAC)	Values calculated manually by the authors of this review
da Silva *et al* (54)	52/Wistar rats/female	Doxorubicin	1.25 mg/kg, ip	Three times a week for 2 weeks	Parameters monitored: aorta-to-left atrial diameter ratio, LVESd, LVEF	Values calculated manually by the authors of this review
Mao *et al* (55)	160/Sprague-Dawley rats/male	Doxorubicin	2 mg/kg, ip	Once a week for 8 consecutive weeks	Parameters monitored: LVEDd, LVESd, LVPWT, interventricular septum thickness (IVST), LVEF, LVFS	Values provided in the manuscript
Deng *et al* (56)	42/Sprague-Dawley rats/male	Doxorubicin (brand name Adriamycin)	2.5 mg/kg, ip (cumulative 15 mg/kg)	6 injections over 2 weeks	Parameters monitored: LV dimensions, LVFS, LVEF	Values calculated manually by the authors of this review
Bertinchant *et al* (57)	45/Wistar rats/male	Doxorubicin	1.5 mg/kg, iv, (cumulative dose 12 mg/kg)	Once a week for up to 8 weeks	Parameters monitored: LVEDd, LVESd and LVFS	Values provided in the manuscript
Sun *et al* (58)	70/Sprague-Dawley rats/male	Doxorubicin	2.5 mg/kg, ip	Once a week for 6 consecutive weeks	Parameters monitored: LVEF, LVEDd, LVESd and LVFS	Values provided in the manuscript
Guerra *et al* (59)	12/SHR rats/male	Doxorubicin	1.5 mg/kg, ip (cumulative dose 13.5 mg/kg)	Once a week for 9 weeks	Parameters monitored: LVEDd, LVESd and LVEF	Values provided in the manuscript
Gao *et al* (60)	90/Wistar albino rats/male	Doxorubicin	2 mg/kg, ip	Every 3 days for 30 days	Parameters monitored: The interventricular septal thickness at diastole, left ventricular internal diameter in diastole and systole, LVPWd at diastole, EF, FS	Values calculated manually by the authors of this review

Table I. Continued.

Publication	No. of animals/rat strain/sex	Anthracycline administered	Anthracycline total dose	Duration	Summary of findings	Calculations
Chen *et al* (61)	60/Sprague-Dawley rats/male	Doxorubicin	2.5 mg/kg, ip	6 injections over 2 weeks	Parameters monitored: LVAW, LVPWT, LVIDd were measured in systole and diastole. EF, FS and LV volume at end-systole and end-diastole	Values calculated manually by the authors of this review
Li *et al* (62)	56/Sprague-Dawley rats/male	Epirubicin	8 mg/kg, ip	Every five days for a total of three injections	Parameters monitored: LV dimensions and wall thickness, EF, FS	Values calculated manually by the authors of this review
Schwarz *et al* (8)	60/Sprague-Dawley rats/female	Doxorubicin (brand name Adriamycin)	2.5 mg/kg, iv	Once a week for 10 weeks	Left ventricular end-systolic and end-diastolic diameters, FS	Values provided in the manuscript
Leontyev *et al* (63)	46/Sprague-Dawley rats/male	Doxorubicin	2.5 mg/kg, ip	Once a week for 9 weeks	LV end-systolic diameter (LVESD) and LV end-diastolic diameter (LVEDD) + FS	Values provided in the manuscript
Merlet *et al* (64)	158/Sprague-Dawley rats/male	Doxorubicin	2.5mg/kg, ip (total 15 mg/kg)	6 injections over 2 weeks	LV end-diastolic and -systolic diameters (LVEDD and LVESD), diastolic posterior wall thicknesses (dPWth). + LV end diastolicand systolic volumes (LVEDV and VESV) to assess LV ejection fraction (LVEF), whereas LV shortening fraction (LVSF)	Values calculated manually by the authors of this review
Ozkanlar *et al* (65)	40/Sprague-Dawley rats/male	Doxorubicin	2.5 mg/kg, iv	Once a week for 3 weeks	Left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS)	Values provided in the manuscript
Hong *et al* (66)	12/Sprague-Dawley rats/male	Doxorubicin (brand name Adriamycin)	5 mg/ week	Once a week for 3 weeks	FS and ejection fraction + interventricular septal dimension diastole; LV internal dimension diastole; LV posterior wall dimension diastole; interventricular septal dimension systole; LV internal dimension systole; LV posterior wall dimension systole	Values provided in the manuscript

Table I. Continued.

Publication	No. of animals/rat strain/sex	Anthracycline administered	Anthracycline total dose	Duration	Summary of findings	Calculations
Teraoka *et al* (67)	75/Wistar rats/male	Doxorubicin (brand name Adriamycin)	1 mg/kg, ip (cumulative dose 15 mg/kg)	15 times over a period of 3 weeks	LV diameter of the systole LVDs + LV diameter of the diastole LVDd. + %fractional shortening	Values provided in the manuscript
Hamed *et al* (68)	130/Wistar rats (Harlan)/male	Doxorubicin	Cumulative dose of 15 mg/kg	3 weeks	LV diameter in systole (LVIDs) LVIDd, LV diameter in diastole; IVSd, intra ventricular septum in diastole LV posterior wall thickness in diastole (LVPWd)	Values provided in the manuscript
Gabrielson *et al* (69)	21/Sprague-Dawley rats/female	Doxorubicin	Cumulative dose of 15 or 7.5 mg/kg	Six or three weekly doses, respectively	Interventricular septum diastole (IVSd) and left ventricular posterior wall thickness at end diastole (PWTED) + LV chamber diameters were measured at the end of diastole (LVEDd) and systole (LVESd). EF%	Values calculated manually by the authors of this review
Yu *et al* (70)	63/Sprague-Dawley rats/male	Doxorubicin	2.5 mg/kg, ip	Once a week for 6 weeks	LV shortening (LVFS) was calculated as ($LVED_d$-LVESd)/LVEDd 9 100, where LVEDd is LV end-diastolic diameter and LVESD is LV end-systolic diameter + LV ejection fraction	Values provided in the manuscript
Bai *et al* (71)	Rats	Doxorubicin	6 injections total 15 mg/kg)	Within 2 weeks	LVEF; LVFS; $LVED_d$ and LVESd	Values provided in the manuscript
Lu *et al* (72)	48/Sprague-Dawley rats/male	Doxorubicin	1 mg/kg on the 2nd and 4th days, 2 mg/kg on the 6th and 8th days, 3 mg/kg on the 10th and 12th days, and 4 mg/kg on the 14th and 16th days, ip		LV internal end-diastolic diameter (diastolic LVID) and the posterior wall end-diastolic thickness (diastolic LVPW) + LV diastolic volume (diastolic LVV) and function indexes (stroke volume, EF and FS)	Values calculated manually by the authors of this review

Table I. Continued.

Publication	No. of animals/rat strain/sex	Anthracycline administered	Anthracycline total dose	Duration	Summary of findings	Calculations
Wachtman *et al* (73)	30/Sprague-Dawley rats/female	Doxorubicin	2.5 mg/kg, iv	Once a week for a total of 6 doses	FS	Values provided in the manuscript
Zhang *et al* (74)	40/Wistar outbred rats/male	Doxorubicin (brand name Adriamycin)	2.5 mg/kg, ip (total 15 mg/kg)	Three times per week for one week. After a two-week interval, administration for another week. These steps were conducted 6 times	The LV end-systolic diameter (LVSD), the LV end-diastolic diameter (LVDD), the LV end-systolic volume (LVSV) and the LV end-diastolic volume (LVDV) + The LV ejection fraction (LVEF) and the LV shortening fraction (LVFS)	Values provided in the manuscript
Chen *et al* (75)	39/ Wister rats/male	Doxorubicin	2.5 mg/kg, ip	Six times for 2 weeks	LV end diastolic diameter (LVEDd), LV end systolic diameter (LVESd) and ejection fraction (EF) + FS + LV systolic pressure (LVSP), LV end diastolic pressure (LVEDP), LV maximum dP/dt and LV minimum dP/dt	Values provided in the manuscript
Ha *et al* (76)	60/Wistar rats/male	Doxorubicin (brand name Adriamycin)	2 mg/kg, iv	Once a week for 2, 4, 6 or 8 weeks, consecutively	LV performance LV dimensions (end-diastolic and end-systolic diameter) + EF	Values calculated manually by the authors of this review
Emanuelo *et al* (77)	40/Sprague-Dawley rats/male	Doxorubicin	2.5 mg/kg, ip (total 15 mg/kg)	Every second day for a period of 2 weeks	LV systolic pressure (LVSP) Diastolic and systolic LV wall thickness, LVEDD, and LVESD were measured + percent LV FS	Values calculated manually by the authors of this review
Lim (78)	52/Sprague-Dawley rats/male	Doxorubicin	2.5 mg/kg, ip	Six times over 2 weeks	LVES dimensions, LVED dimensions, LVFS	Values provided in the manuscript
Hydock *et al* (79)	147/Sprague-Dawley rats/male	Doxorubicin	10 mg/kg, ip	Acute administration (bolus injection)	SWT during systole (SWs) and diastole (SWd), PWT and PWT during diastole (PWd), LVEDd, LVESd, FS	Values calculated manually by the authors of this review
Xiang *et al* (80)	37/Sprague-Dawley rats/male	Doxorubicin	2.5 mg/kg, ip	Once a week for 6 weeks	LVEDd and LVESd + LV FS (%)	Values provided in the manuscript

Table I. Continued.

Publication	No. of animals/rat strain/sex	Anthracycline administered	Anthracycline total dose	Duration	Summary of findings	Calculations
Kenk *et al* (81)	94/Sprague-Dawley rats/male	Doxorubicin (brand name Adriamycin)	2.5 mg/kg, ip (total 15 mg/kg)	6 injections over 2 weeks	LV internal diameter (LV diastolic and systolic dimensions; LVDD and LVSD), LV posterior wall (LVPW), and intra-ventricular septum (IVS) thickness at end-diastole and peak systole. →LV volume in diastole and systole (LVDV, LVSV), stroke volume (SV), EF, FS, and LV mass	Values provided in the manuscript
Katona *et al* (82)	23/Adult Wistar rats/male	Doxorubicin (brand name Adriamycin)	2.5 mg/kg, ip	Three times a week for 2 weeks	Parameters monitored: LVDDd and LVSDd, FS, LAD, AOD	Values provided in the manuscript
Hydock *et al* (83)	49/Sprague-Dawley rats/female	Doxorubicin	1.5 mg/kg i.p of (cumulative 15 mg/kg)	Once a day for 10 consecutive days	Septal wall thickness at systole (SWs) and diastole (SWd), posterior wall thickness at systole (PWs) and diastole (PWd), LVDs and LVDd, and FS	Values provided in the manuscript
Hou *et al* (84)	40/Wistar rats/male	Doxorubicin (brand name Adriamycin)	2.5 mg/kg, ip	6 times for 2 weeks	LV dimensions [end-diastolic diameter (LVDd) and end systolic diameter (LVDs)] + % FS of the LV	Values provided in the manuscript
Hydock *et al* (85)	74/Sprague-Dawley rats/male	Doxorubicin	1 mg/kg, ip (total 10 mg/kg)	Once a day for 10 consecutive days	Septal wall thickness at systole (SWs) and diastole (SWd), posterior wall thickness at systole (PWs) and diastole (PWd), LVDs and LVDd. + FS, LV mass and relative wall thickness (RWT).	Values provided in the manuscript
Koh *et al* (86)	33/Wistar rats/male	Doxorubicin (brand name Adriamycin)	2 mg/kg, iv	Once a week for 8 weeks	LV dimensions (the LVDd, LVDs, the intraventricular septal thickness, and the LV posterior wall thickness) + % FS of LV atrial natriuretic peptide; brain natriuretic peptide	Values provided in the manuscript

Table I. Continued.

Publication	No. of animals/rat strain/sex	Anthracycline administered	Anthracycline total dose	Duration	Summary of findings	Calculations
Carresi *et al* (87)	40/Wistar rats/male	Doxorubicin	2.5 mg/kg, ip	6 times for 2 weeks	LVESd; LVEDd; IVSs; IVSd, LVPWs and LVPWd; EF; FS	Values provided in the manuscript
Ma *et al* (88)	190/Wistar rats/male	Doxorubicin	2.5 mg/kg, ip	6 times for 2 weeks	LVEDD) and LVESD + FS + EF	Values provided in the manuscript
Zhang *et al* (89)	26/Sprague-Dawley rats/male	Doxorubicin	4 mg/kg, ip (cumulative dose 16 mg/kg)	Twice per week for 2 weeks	Diastolic interventricular septum thickness (IVSTd), systolic interventricular septum thickness (IVSTs), + EF + FS	Values calculated manually by the authors of this review
Sun *et al* (90)	32/Sprague-Dawley rats/male	Doxorubicin	20 mg/kg, ip 5.0 mg/kg, iv	Acute administration (single dose)	(LVEF) from EDV and ESV, + EDV and ESV + LVFS	Values provided in the manuscript
Zhu *et al* (91)	50/Adult Sprague-Dawley rats/male	Doxorubicin	2 mg/kg/week	6 weeks	Ejection fraction	Values provided in the manuscript
Croteau *et al* (92)	12/ Fisher rats/male	Doxorubicin	2 mg/kg, iv	Once a week for 6 weeks	Left ventricular function Left ventricle ejection fraction	Values provided in the manuscript
Ikegami *et al* (93)	14/Sprague-Dawley/NM	Doxorubicin	2.5 mg/kg, ip	3 times a week for 2 to 6 weeks	LVDd and LVFS + FS	Values provided in the manuscript
Hiona *et al* (94)	24/Sprague Dawley rats/female	Doxorubicin	Cumulative dose of 25 mg/kg, ip	Once a week for 6 weeks	LVFS	Values provided in the manuscript
Tang *et al* (95)	40/Sprague-Dawley rats/male	Doxorubicin	2.5 mg/kg, ip	Once a day for a total of 6 times	Parameters monitored: LVEF, LVIDd, LVIDs, LVPWd, LVPWs, left ventricle % EF, and left ventricle % FS	Values provided in the manuscript
Migrino *et al* (96)	31/Sprague Dawley rats/male	Doxorubicin	2.5 mg/kg, iv	Once a week for 10 or 12 weeks	FS monitored	Values provided in the manuscript
Liu *et al* (97)	24/Sprague-Dawley rats/male	Doxorubicin (brand name Adriamycin)	Each dose consisted of 1, 1, 2, 2, 3 and 3 mg/kg, ip (cumulative dose 12 mg/kg)	At 1st, 3rd, 5th, 7th, 9th and 11th day, respectively	Parameters monitored: interventricular septum thickness of systolic, IVSd, LVIDd, LVISd, LVPW, LVPWd, EF, FS	Values provided in the manuscript
Liu *et al* (98)	120/Sprague Dawley rats/NM	Doxorubicin	3.3 mg/kg, iv	Once a week for 4 weeks		Values provided in the manuscript

LV, left ventricular; LVEF, LV ejection fraction; LVFS: LV fractional shortening; BNP, brain natriuretic peptide; PWT, posterior wall thickness; AWT, anterior wall thickness; SWT, septal wall thickness; BP, blood pressure; HR, heart rate; LVSP, LV systolic pressure; LVDP, LV diastolic pressure; LVEDd, LV end-diastolic diameter; LVESd, LV end-systolic diameter; LVEDV, LV end-diastolic volume; LVIDd, LV internal diastolic diameter LVISd, LV internal systolic diameter; LVPWs, LV systolic wall thickness; LVPWd, LV diastolic wall thickness; IVSd, intraventricular septum in diastole; LAD, left atrial diameter; AOD, aortic diameter; ip, intraperitoneally; iv, intravenously; NM, not mentioned; SD, Sprague-Dawley.

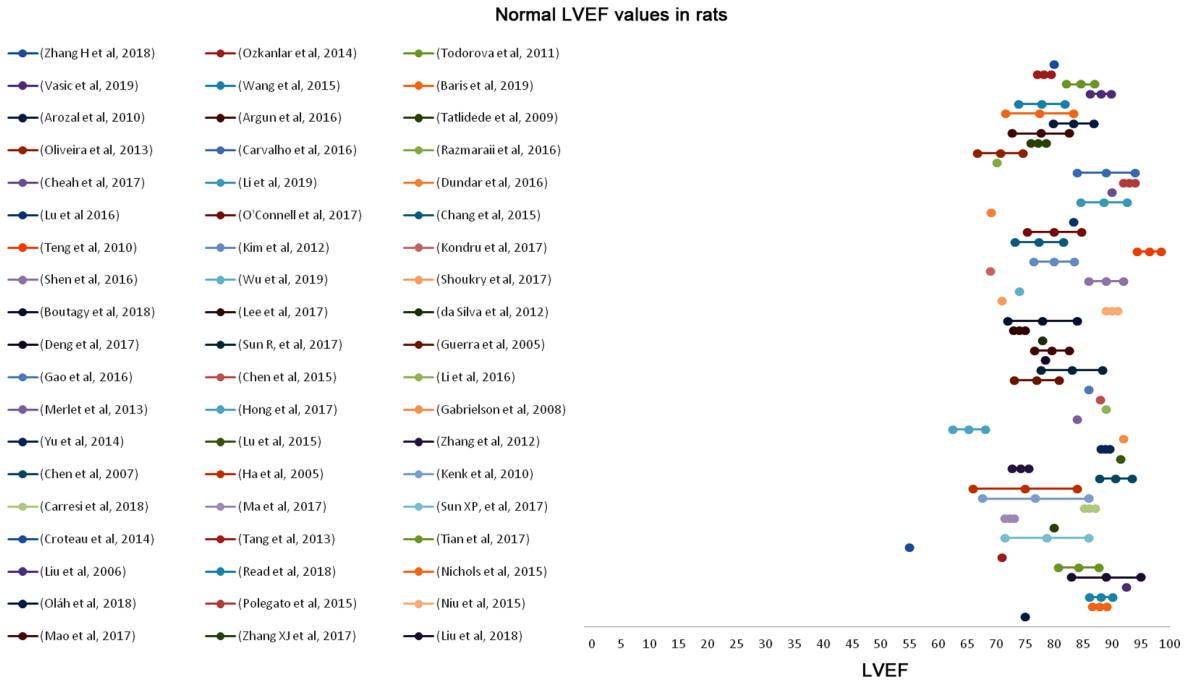

Figure 2. Normal (baseline) LVEF values in rats before anthracycline administration as reported in 57 relevant studies reviewed in the present report. LVEF, left ventricular ejection fraction.

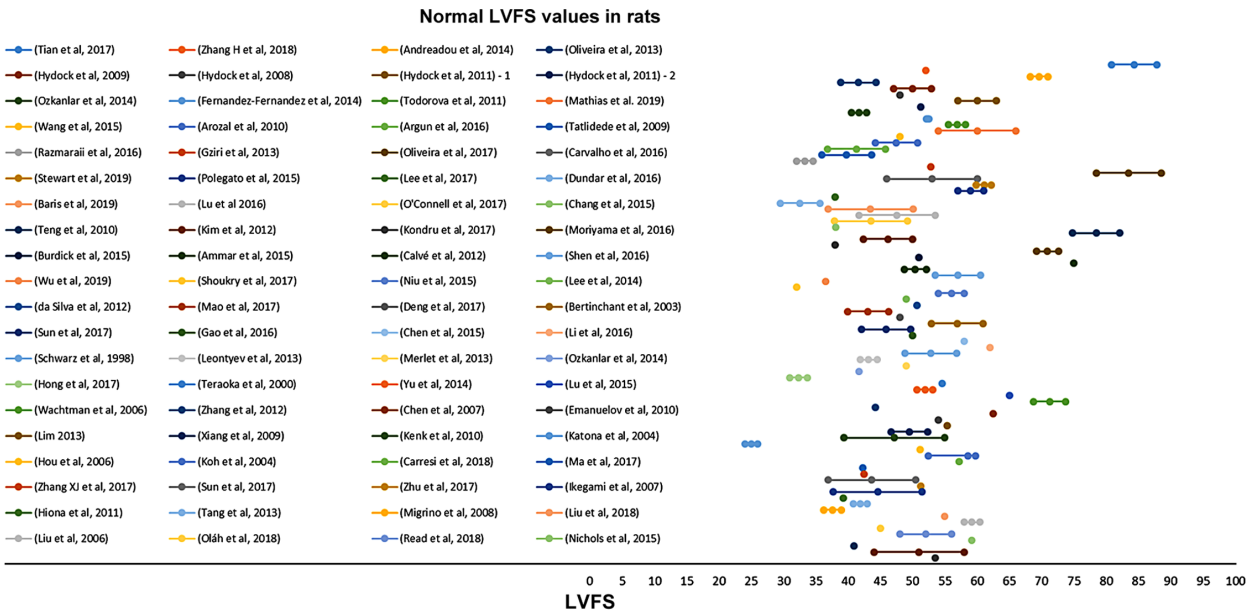

Figure 3. Normal (baseline) LVFS values in rats before anthracycline administration as reported in 80 relevant studies reviewed in the present report. LVFS, left ventricular fractional shortening.

largely based on recommendations. The American Society of Clinical Oncology, for example, recommends active screening and prevention of modifiable cardiovascular risk factors, such as tobacco use, high blood pressure, high cholesterol, alcohol use, obesity and physical inactivity (101). A well characterized animal model for defining cardiotoxicity due to chemotherapy and the treatment thereof is of great importance for clinical practice, as it will enable physicians to base their decisions not only on epidemiology but also on observations developed using concrete data from animal studies.

In the present review, the range of the main echocardiographic indices, namely EF and FS, used in describing anthracycline cardiotoxicity in rats was summarized along with the normal values of the said indices presented in the respective studies. In the graphic representation, it seems that normal and suppressed values due to anthracyclines administration for the two echocardiographic indices are well separated. This provides the first evidence for the possibility of setting a cut-off point for defining anthracycline cardiotoxicity in rats with an in-depth future meta-analysis.

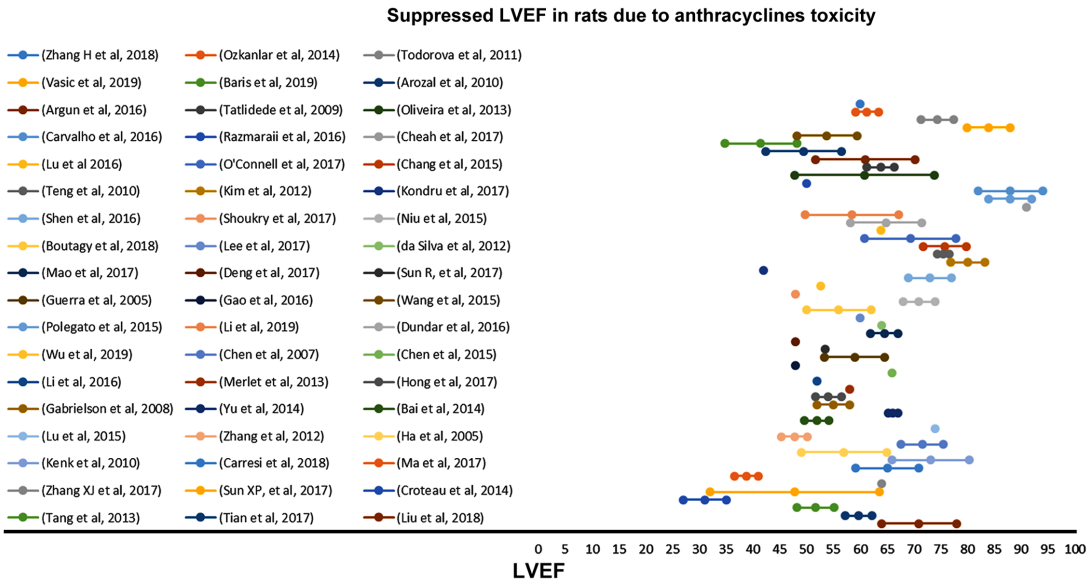

Figure 4. Suppressed LVEF values in rats due to anthracycline toxicity as reported in 54 relevant studies reviewed in the present report. LVEF, left ventricular ejection fraction.

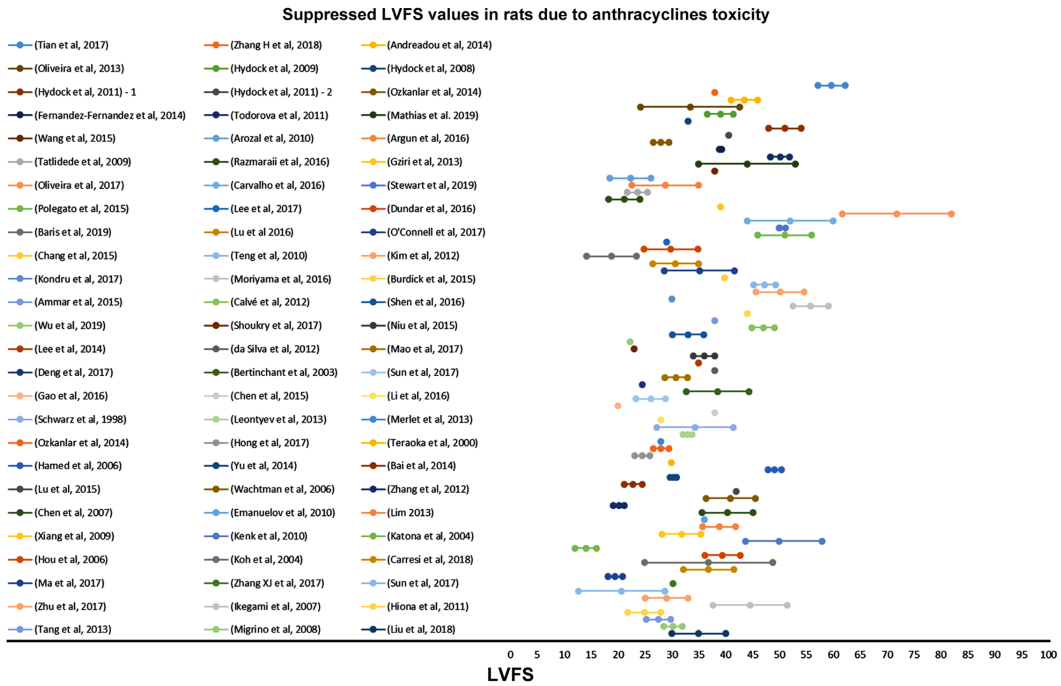

Figure 5. Suppressed LVFS values in rats due to anthracycline toxicity as reported in 78 relevant studies reviewed in the present report. LVFS, left ventricular fractional shortening.

In the current study a wide range of EF and FS decline due to anthracycline administration was observed. However, the trends of the said decline are easily identified, especially for FS values, thus rendering the establishment of minimum cut off values of decline feasible. The question remains, as it has also been identified for humans, whether the absolute suppressed values of EF and FS, combined or separately, or the % suppression caused by anthracyclines should be used to describe cardiotoxicity, and which of the two approaches could be more effective in prevention. In our study, it seems that setting a range for % suppression of EF and FS could be more efficient in identifying early cardiotoxicity by counteracting the intra-individual variation of the absolute values.

In the current in depth review analysis, we did not identify differences between rat strains in terms of suppressed EF and FS values due to anthracycline administration. This is an interesting finding as it seems that the usual strains used in rat studies are equally prone to the cardiotoxic anthracycline potential. In animal models of genetically programmed hypertension and heart failure, it is found that doxorubicin administration did not lead to lower myocardial contractility compared to non-genetically modified strains (102). In addi-

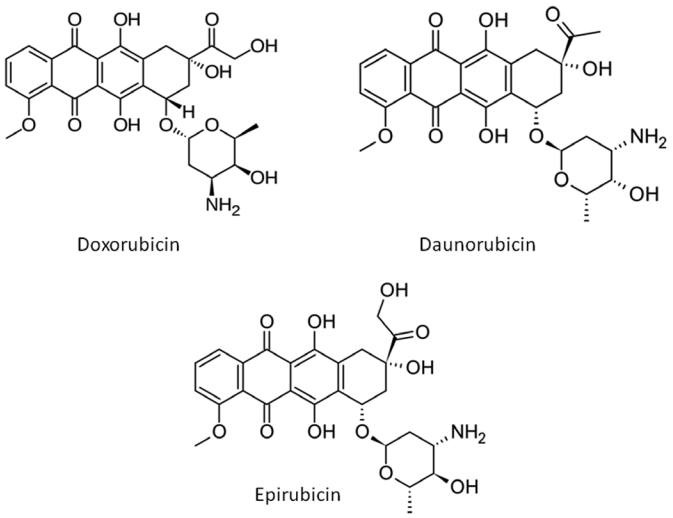

Figure 6. Chemical structures of the three anthracyclines used to induce cardiotoxicity in the studies reviewed in the present report.

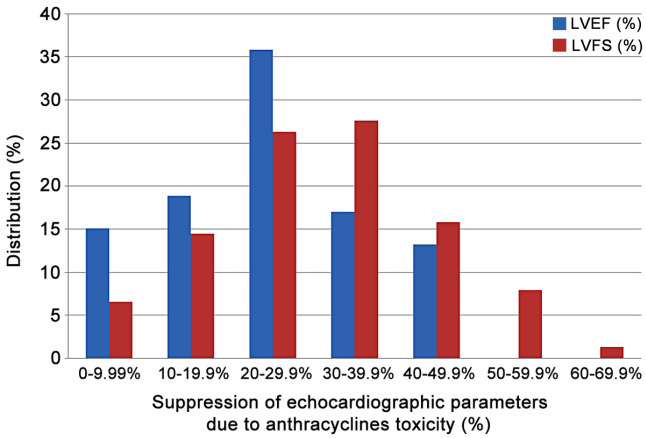

Figure 7. Percentiles distribution of % suppression of LVEF and LVFS due to anthracycline toxicity as mentioned in the studies reviewed in the present report. LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening.

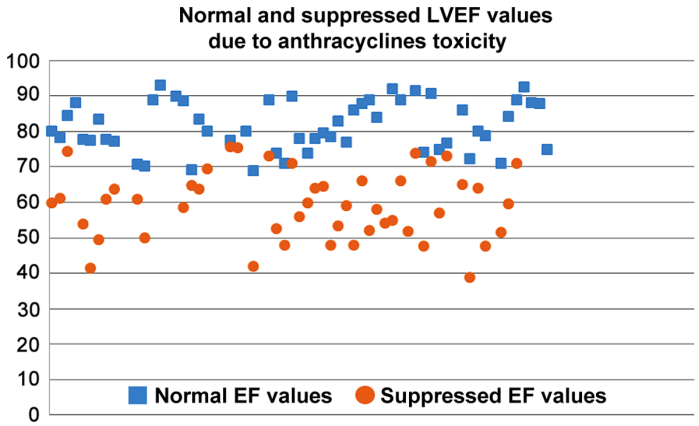

Figure 8. Scatter plot of normal (baseline) and suppressed LVEF values in rats due to anthracycline toxicity as reported the studies reviewed in the present report. LVEF, left ventricular ejection fraction.

tion, in the current systematic review, acute and chronic anthracyclines cardiotoxicity models were found equally potent in inducing cardiotoxicity based on evaluated echocardiographic indices.

Currently, when assessing chemicals toxicity, cardiac effects if monitored and detected in animal studies, mainly on the tissue level, are considered by the authorities, but cardiotoxicity, as such, is not described as a separate hazard class of

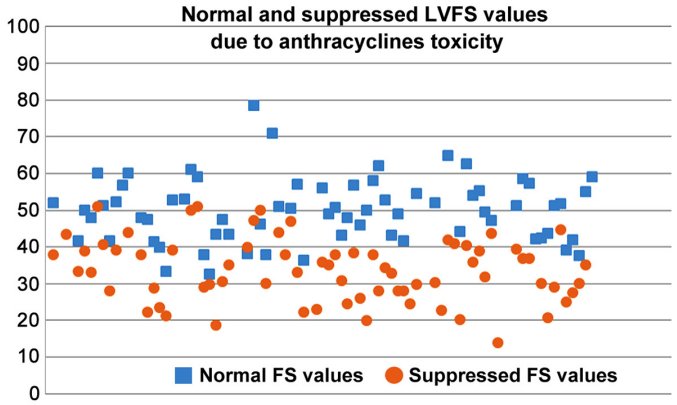

Figure 9. Scatter plot of normal (baseline) and suppressed LVFS values in rats due to anthracycline toxicity as reported in the studies reviewed in the present report. LVFS, left ventricular fractional shortening.

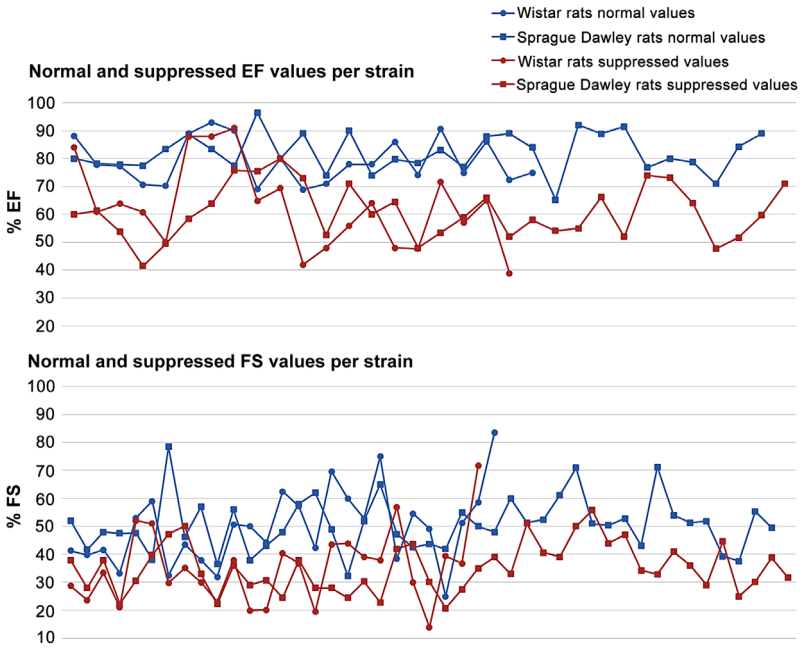

Figure 10. Normal and suppressed LVEF and LVFS values for the two main rat strains used in the studies reviewed in the present report. LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening.

chemical substances through the available regulations, both at a European level and world-wide. Therefore, chemicals other than pharmaceutical agents are recognised to be cardiotoxic after having exerted such deleterious effects on humans, based on epidemiological studies. In a previous review of our research team, the cardiac pathology and function impairment due to exposure to pesticides revealed that several cardiovascular complications have been reported in animal models including electrocardiogram abnormalities, myocardial infarction, impaired systolic and diastolic performance and histopathological findings, such as haemorrhage, vacuolization, signs of apoptosis and degeneration (103). In addition, there is evidence that short and/or long-term exposure to anabolic androgenic steroids is linked to a variety of cardiovascular complications which could be identified by using echocardiography or biochemical markers (10,104,105). The published data suggest clearly that there is a need to establish regulatory criteria for assessing cardiotoxicity as an inherent property of a chemical substance well in advance, and characterize the risk of exposure to such chemicals through a well-developed regulatory network based on animal models, as is the case for other human health hazard classes, such as carcinogenicity. Regulatory established criteria will enable international organizations to early identify cardiotoxic effects and classify chemicals in order to avoid long-term cardiovascular complications. Specific classification criteria should be developed based on anatomical, histopathological, echocardiographic and biochemical criteria in animals developed in a way that could exclude confounding factors in the development of the observed cardiotoxicity. The results of the present study are promising in identifying echocardiographic criteria in rats for the establishment of cardiotoxicity. Further studies and meta-analyses are needed in order to evaluate other species, commonly used in research, and explore the possibility of early recognizing the onset of cardiotoxicity, possibly through monitoring of biochemical markers based on understanding of the mode of action.

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Availability of data and materials

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Authors' contributions

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Ethics approval and consent to participate

Not applicable

Patients consent for publication

Not applicable

Competing interests

DAS is the Editor-in-Chief for the journal, but had no personal involvement in the reviewing process, or any influence in terms of adjudicating on the final decision, for this article. The positions and opinions presented in this article are those of the authors (NG, GENK, JLCMD) alone and are not intended to represent the views or any official position or scientific works of the European Agencies EFSA and ECHA. The other authors declare that they have no competing interests.

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