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Review

Disulfiram Repurposing Across Therapeutic Indications: Molecular Mechanisms, Evidence Appraisal, and a Systematic Review of Its Use in Lyme Borreliosis

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Abstract

Disulfiram (tetraethylthiuram disulfide, DSF), licensed for alcohol use disorder since 1951, is among the most studied drug-repurposing candidates and has been prescribed off-label for Lyme borreliosis since 2016. This hybrid review asks what supports that practice, appraising the Lyme evidence against disulfiram's record in other repurposing indications. We provided a narrative review of DSF and its bioactive metabolites' pharmacology, molecular targets, and toxicology. We also included a separate section systematically reviewing DSF in Lyme borreliosis following the PRISMA 2020 Guide. PubMed, Europe PMC, CENTRAL, bioRxiv, medRxiv, and ClinicalTrials.gov were searched from inception, while risk of bias was assessed using ROBINS-I, RoB 2, and SYRCLE; certainty was analyzed using GRADE. DSF is a prodrug acting through twenty-one documented targets, yet only alcohol use disorder rests on regulatory-grade evidence; oncology results are contradictory, and other indications remain preclinical. Nine primary studies addressed Lyme borreliosis, with five human reports contributing 96 participant entries and 93 unique individuals. Preclinical findings conflict, the largest dataset is uncontrolled, and the only randomized trial stopped early. Evidence on DSF repurposing shows strong preclinical support and small or inconsistent controlled results; a few well-powered randomized trials comparing it to standard care reported no clear benefits, but increased toxicity. In Lyme borreliosis, certainty of DSF benefits is very low, and certainty of dose-dependent harm is moderate, including one fatal outcome. No human study measured a microbiological endpoint, and all preclinical work used *B. burgdorferi sensu stricto*, leaving the evidence indirect for European genospecies.

Keywords: disulfiram; drug repurpose; diethyldithiocarbamate; molecular targets; systematic review; PRISMA 2020; GRADE; Lyme borreliosis; *Borrelia burgdorferi*; drug safety; evidence-based medicine

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1. Introduction

Disulfiram (DSF, tetraethylthiuram disulfide, Antabuse) was first approved in 1951 as an aversive treatment for alcohol use disorder and has remained approved for this sole purpose ever since [1]. Its pharmacological effects go beyond aldehyde dehydrogenase inhibition. As a thiol-reactive electrophile whose main metabolite chelates divalent metals,

it interacts with various targets across different disease areas. This has made it one of the most extensively studied drugs for medical repurposing [2,3].

DSF has been proposed for various conditions, including cancer, inflammation, sepsis, coronavirus infection, HIV latency reversal, cocaine use disorder, obesity, metabolic syndrome, nickel dermatitis, and, since 2016, Lyme borreliosis [3–5]. The evidence supporting these claims varies widely, ranging from randomized controlled trials to single uncontrolled case series. These different levels of evidence are rarely presented together, making it difficult to gauge the strength of each claim. Therefore, the present review has three main objectives:

(i) First, it identifies molecular targets and mechanistic pathways of DSF and its bioactive metabolites that underlie their broad pharmacological potential and toxicity;
(ii) Second, it evaluates the evidence supporting each DSF repurposing indication and states the highest level of evidence for each;
(iii) Third, it systematically reviews the literature regarding DSF use in Lyme borreliosis, emphasizing areas with the largest gap between clinical practice and evidence, especially where patients are treated outside controlled trials.

2. Pharmacology of Disulfiram

2.1. DSF Biotransformation in the Human Body

Disulfiram acts as a prodrug, and little of the administered dose reaches systemic circulation unchanged [6,7]. After oral intake, approximately 80–90% is absorbed, and glutathione and other free thiols reduce its disulfide bond in the gut lumen, erythrocytes, and liver, producing two molecules of diethyldithiocarbamate (DDC) [6,7]. DDC is central to the process: it is chemically unstable, binds divalent metals, and serves as the substrate for all subsequent pathways [3,7]. DDC is processed through four pathways: it decomposes non-enzymatically at gastric pH into carbon disulfide and diethylamine [7,8]; forms a glucuronide conjugate for renal excretion [6,7]; chelates metals such as copper, zinc, and manganese to create bis(dithiocarbamate)-metal complexes, with the copper complex CuET acting as the main active species in tissues [3]; and is S-methylated by thiol methyltransferase to form S-methyl-DDC, which hepatic cytochrome P450 enzymes further oxidize into sulfoxide and sulfone [9,10]. This oxidized carbamate—not disulfiram or DDC—carbamoylates the active-site cysteine of aldehyde dehydrogenase, producing the alcohol-aversive effect [9,10] (Table 1).

Table 1. Metabolic cascade of disulfiram and its metabolites: bioactivity, molecular targets, and mechanistic pathways.

Step	DSF/ Metabolites	Route	Bioactivity	Reference
0	DSF	Administered orally, 250–500 mg/day; ~80–90% absorbed	Direct thiol electrophile: gasdermin D Cys191 modification blocking pyroptosis; mixed disulfides with bacterial thiols. Little intact drug reaches systemic circulation	[5–7]
1	Diethyldithiocarbamate (DDC)	Reduction of the disulfide bond by glutathione and free thiols in gut, erythrocytes, and liver; two molecules per parent molecule	Central metabolite: metal chelator and electrophile; substrate for all downstream branches; inhibits dopamine beta-hydroxylase	[1,6,7]
2a	Carbon disulfide + diethylamine	Non-enzymatic decomposition of DDC, favored at gastric pH; CS_2 partly exhaled and measurable in blood	Antimicrobial and small enough to traverse biofilm matrix (hypothesis); also implicated in neurotoxicity and hepatotoxicity	[7,8,11]
2b	DDC-glucuronide	Hepatic glucuronidation	Inactive; principal route of urinary elimination	[6,7]

Table 1. *Cont.*

Step	DSF/ Metabolites	Route	Bioactivity	Reference
2c	CuET and other bis(DDC)-metal complexes	Chelation of Cu, Zn, Mn by DDC	CuET accumulates in tissue and immobilizes the p97 adaptor NPL4; Mn/Zn complexes proposed to deprive B. burgdorferi of essential cofactors	[3,12,13]
3a	S-methyl-DDC (MeDDC)	S-methylation of DDC by thiol methyltransferase	Intermediate; not itself an effective ALDH inhibitor	[9,10]
3b	MeDDC sulfoxide, then sulfone	Cytochrome P450-mediated sulfoxidation of MeDDC in the liver	Proximate inhibitor of aldehyde dehydrogenase: carbamoylates the active-site cysteine, producing irreversible inhibition and the disulfiram-ethanol reaction	[9,10]

Molecular Target	Molecular Event	Therapeutic Field	Reference
Aldehyde dehydrogenase inhibition	Irreversible inhibition of ALDH1/ALDH2; acetaldehyde accumulates after ethanol exposure, producing the disulfiram-ethanol reaction	Alcohol use disorder (licensed mechanism); also, the source of the principal safety hazard	[1,2,14]
Metal chelation and complex formation	DDC binds Cu, Zn, Mn, and other divalent cations; CuET is the pharmacologically active species and accumulates in tissue	Oncology; proposed antimicrobial mechanism via competition for bacterial metal cofactors	[3,12,13]
Thiol–disulfide exchange	Electrophilic attack on free cysteine thiols, forming mixed disulfides and disabling thiol-dependent enzymes	Broad; underlies both antimicrobial and off-target toxicity	[11,15]
Proteasome and p97/NPL4 segregase	CuET immobilizes the p97 adaptor NPL4, causing protein-turnover arrest and a heat-shock-like stress response	Oncology; also cited as a candidate antimicrobial route	[3]
Gasdermin D and pyroptosis	Covalent modification of Cys191 (human)/Cys192 (mouse) blocks GSDMD pore formation, preventing IL-1beta release and pyroptotic death at nanomolar concentrations	Inflammation, sepsis, COVID-19; relevant to symptom change independent of any antimicrobial effect	[5]
Dopamine beta-hydroxylase inhibition	Reduced conversion of dopamine to noradrenaline	Cocaine use disorder; plausible contributor to the psychiatric adverse-event profile	[1]
Cytochrome P450 inhibition	Inhibition of hepatic CYP-mediated metabolism, raising concentrations of co-administered substrates such as warfarin and phenytoin	Drug-interaction hazard across all indications	[2,16]
Carbon disulfide generation	CS_2 is produced as a metabolite in measurable blood concentrations; antimicrobial and small enough to diffuse through biofilm matrix	Proposed mechanism for activity against sessile organisms	[8,11]
20S proteasome and POH1	DSF/Cu partially binds the 20S proteasome and inhibits chymotrypsin-like activity; the deubiquitinase POH1 in the proteasome lid is a further proposed site; poly-ubiquitinated IκB, p27Kip1, Bax and c-Myc accumulate	Oncology: apoptosis induction and 74% tumor growth inhibition in breast cancer xenografts	[17–19]
NF-κB signalling	Blocking IκB degradation prevents nuclear translocation of NF-κB, removing a survival, stemness, and angiogenesis signal	Oncology; also relevant to anti-inflammatory activity	[20]
ALDH1A1 in cancer stem cells	Depletion of the ALDH-high stem-like fraction with suppression of Nanog and Oct4; reversal of platinum resistance	Oncology: glioblastoma stem cells, ovarian and testicular germ-cell tumors	[21]
DNA methyltransferase 1	Non-nucleoside, dose-dependent inhibition of DNMT1 on hemi-methylated substrate; global 5-methylcytosine falls and silenced genes including APC are reactivated	Oncology: epigenetic reactivation in prostate cancer lines and xenografts	[22]
MMP-2 and MMP-9	Suppressed type IV collagenase expression and activity	Oncology: reduced invasion in osteosarcoma cells	[23]
ATF/CREB	Metal-dependent inhibition of ATF/CREB DNA binding	Oncology: melanoma growth inhibition in vitro, in mice and in one patient	[24]

Table 1. *Cont.*

Molecular Target	Molecular Event	Therapeutic Field	Reference
FROUNT	Direct binding to FROUNT blocks its interaction with the CCR2 and CCR5 cytoplasmic domains	Oncology and immunology: suppressed macrophage chemotaxis and tumor-promoting macrophage accumulation	[25]
PD-L1 stabilization	DSF/Cu stabilizes PD-L1 expression	Oncology: an immunosuppressive effect running counter to the other immune mechanisms	[26]
Autophagy and hepatic energy metabolism	Potent activation of autophagy in cell culture; convergent hepatic transcriptomic and proteomic signatures in lipid and energy metabolism, redox and detoxification	Metabolic disease: weight normalization and improved insulin sensitivity in obese mice	[27]
Mitochondrial matrix proteins	Induction of protein insolubility specifically within the mitochondrial matrix, demonstrated by phenotypic imaging screens	Mechanism of off-target toxicity, distinct from ALDH inhibition	[28]

The DSF cascade has three clinically important features. Inhibition of aldehyde dehydrogenase is irreversible, so activity resumes only through new enzyme production, and alcohol sensitivity can persist for up to two weeks after the last dose [1,6,7]. Because all key steps occur in the liver, any degree of hepatic impairment is a contraindication and suggests that hepatotoxicity results from metabolite buildup rather than the parent drug [7,29–31]. Because active metabolites are produced through both enzymatic and non-enzymatic pathways, the oral dose is an imperfect indicator of exposure at any specific target. This complexity partly explains why dose–response relationships in Lyme research are difficult to interpret [7,11,16].

2.2. Molecular Targets of DSF and Its Metabolites

The species generated by that cascade act on several unrelated targets, which is why one molecule appears across the oncology, inflammation, and infectious-disease studies [2,3,5]. Metal chelation and thiol reactivity, rather than aldehyde dehydrogenase inhibition, account for most of the non-alcohol pharmacology [3,5].

Table 1 also consolidates the documented targets across all indications. Three chemical facts underline the entire list: diethyldithiocarbamate chelates copper, zinc, and manganese; the parent disulfide carbamoylates or forms mixed disulfides with reactive cysteine residues; and both the parent and metabolites partition into tissues. This promiscuity explains why disulfiram appears across oncology, inflammation, virology, metabolic, and anti-infective studies, and why its clinical specificity has been so difficult to establish (Figure 1).

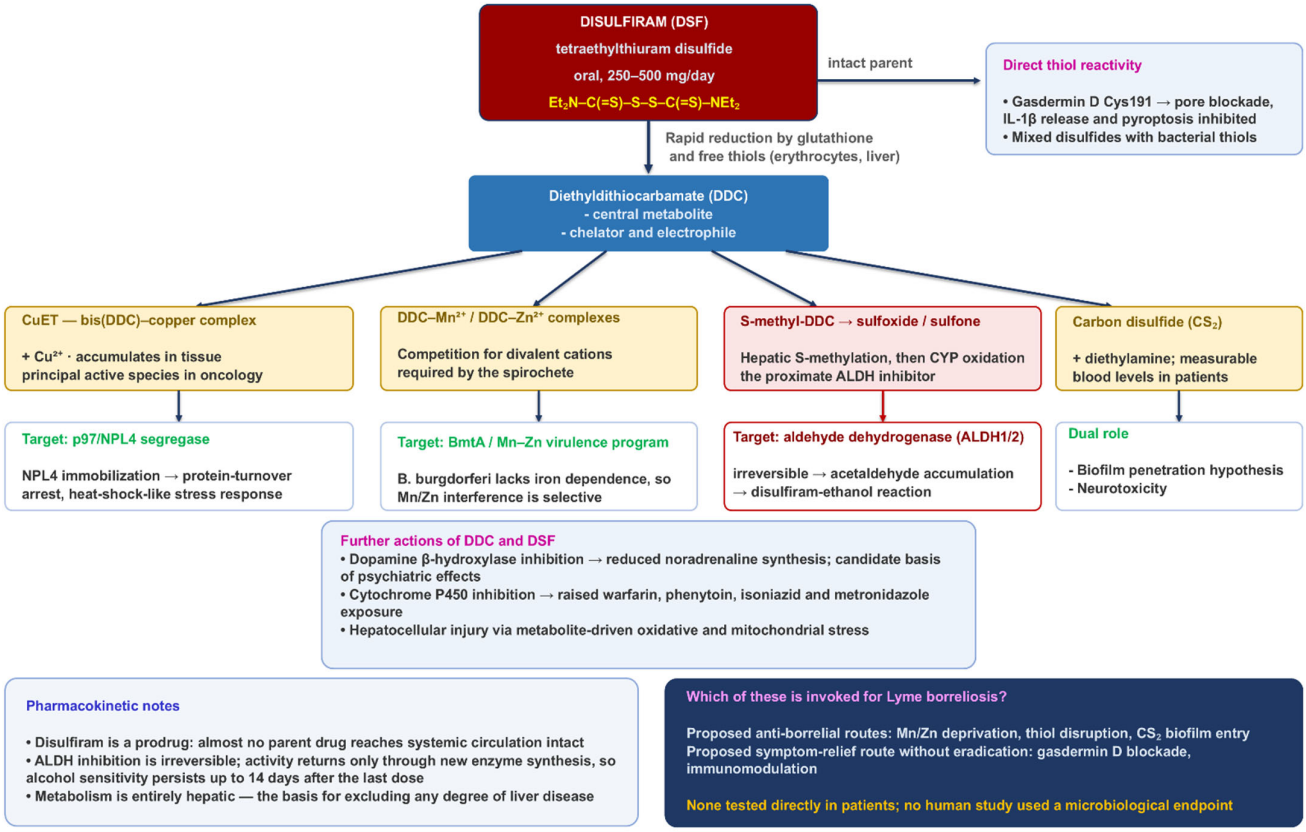


Figure 1. DSF biotransformation and molecular targets of its bioactive metabolites. DSF is a prodrug: it is reduced to diethyldithiocarbamate, which chelates divalent metals and is further converted to carbon disulfide and, via S-methylation and cytochrome P450 oxidation, to the carbamate sulfoxide and sulfone that irreversibly inhibit aldehyde dehydrogenase.

3. Evidence for Disulfiram Repurposing Across Indications

3.1. Levels of Evidence

The strength of evidence for DSF repurposing varies significantly, with only the first backed by regulatory-grade controlled studies. Table 2 provides the strongest studies available in each field.

Table 2. The highest level of evidence attained for each proposed indication.

Indication	The Highest Level of Evidence	Principal Quantitative Result	Status	Reference
Alcohol use disorder	Randomized controlled trials; licensed indication	Aversive efficacy established; 250–500 mg/day	Licensed; second-line behind naltrexone and acamprosate	[1,2,32,33]
Oncology—metastatic NSCLC	Randomized double-blind phase IIb	Disulfiram 40 mg three times daily added to cisplatin and vinorelbine: median survival 10 vs. 7.1 months (n = 40)	Positive but small and unreplicated	[34]
Oncology—recurrent glioblastoma	Randomized phase II/III	No survival benefit (median 5.5 vs. 8.2 months); grade ≥ 3 adverse events 34% vs. 11%; serious adverse events 41% vs. 16%; 24% discontinued	Negative, with excess toxicity	[35]
Oncology—newly diagnosed glioblastoma	Phase I/II dose-finding	500 mg daily tolerable; 1000 mg daily produced dose-limiting toxicity	Dose-defining only	[36]
Oncology—population signal	Observational cohort	Lower cancer mortality among continuing disulfiram users	Hypothesis-generating	[3]

Table 2. *Cont.*

Indication	The Highest Level of Evidence	Principal Quantitative Result	Status	Reference
Cocaine use disorder	Randomized controlled trials	Reduced cocaine use with behavioral therapy in some populations; results inconsistent across trials	Off-label, unresolved	[37]
HIV latency reversal	Phase 2 dose escalation	Effect on the latent reservoir; pharmacodynamic endpoint only	No efficacy trial	[38]
Inflammation and sepsis	Preclinical	Gasdermin D Cys191 modification blocks pore formation at nanomolar concentrations, preventing IL-1β release and pyroptosis	Preclinical; basis of COVID-19 trials	[5]
COVID-19	Registered trial	Entered trials on the pyroptosis-blocking rationale; no published efficacy result	Unreported	[39]
Tuberculosis	In vitro	Dithiocarbamates active against growing and non-replicating *M. tuberculosis*	Preclinical	[40]
SARS-CoV and MERS-CoV	Enzymatic assays	Inhibition of papain-like proteases by allosteric and competitive modes, respectively	Preclinical	[41]
Obesity and metabolic syndrome	Animal, mechanistic	Reduced feeding efficiency, lower body weight, higher energy expenditure, improved glucose tolerance and insulin sensitivity in obesogenic-diet mice	Preclinical; human trials registered	[27,42]
Nickel dermatitis	Small double-blind study	Chelation-based benefit; source of comparator hepatotoxicity estimates	Historical indication	[43]
Lyme borreliosis	Randomized pilot without placebo comparator	Terminated early; 5/9 discontinued for adverse reactions	Off-label, efficacy unproven—appraised in Section 5	[11,16,44,45]

3.2. Alcohol Use Disorder

This is the only licensed indication of DSF, supported by controlled trials and 70 years of practice; national guidance nevertheless positions it as second-line behind naltrexone and acamprosate, restricted to patients who understand the alcohol-interaction risk and have no contraindications, at 250–500 mg/day [1,2,32,33].

3.3. Oncology

Extensive preclinical activity and an observational signal of improved cancer mortality among continuing users [3] are offset by inconsistent controlled results. In a randomized, double-blind phase IIb trial in metastatic non-small cell lung cancer, adding disulfiram 40 mg three times daily to cisplatin and vinorelbine was well tolerated and associated with longer median survival (10 vs. 7.1 months, $n = 40$) [34]. In contrast, a randomized phase II/III trial in recurrent glioblastoma found that adding disulfiram 400 mg daily plus copper to alkylating chemotherapy produced no survival benefit (median overall survival 5.5 vs. 8.2 months) but significantly more grade 3 or higher adverse events (34% vs. 11%) and serious adverse events (41% vs. 16%), with 24% discontinuing due to adverse effects [35]. A phase I/II study in newly diagnosed glioblastoma established 500 mg daily as tolerable, whereas 1000 mg produced dose-limiting toxicity [36].

Mechanistically, DSF's anticancer potential does not rest on aldehyde dehydrogenase inhibition. The copper complex CuET immobilizes NPL4, the cofactor of the p97 segregase, leading to NPL4 aggregation, arrest of protein turnover, and a proteotoxic heat-shock response; this was identified as the dominant anticancer target [3]. Convergent effects are reported on the 20S proteasome and the lid deubiquitinase POH1, with accumulation of polyubiquitinated IκB, p27Kip1, Bax, and c-Myc, 74% growth inhibition of MDA-MB-231 xenografts, and blockade of NF-κB nuclear translocation [17–20].

Additional mechanistic pathways have been documented: depletion of the ALDH-high stem-like fraction, with suppression of Nanog and Oct4 [21]; non-nucleoside DNMT1 inhibition, leading to global demethylation and reactivation of silenced genes [22]; suppression of MMP-2 and MMP-9, reducing invasion [23]; metal-dependent inhibition of ATF/CREB [24]; and FROUNT blockade, which suppresses macrophage chemotaxis and the accumulation of tumor-promoting macrophages [25]. Not all immune effects favor the host: in hepatocellular carcinoma, DSF/Cu stabilizes PD-L1, an immunosuppressive effect that runs counter to the others [26].

Clinical findings vary, but the most significant insight in the repurposing field is the contrast between two randomized trials. The phase IIb trial for metastatic non-small cell lung cancer showed longer median survival when DSF was combined with chemotherapy [34]. In contrast, the larger randomized trial for recurrent glioblastoma found no survival advantage, a threefold increase in grade 3 or higher adverse events, and noted that 25% of patients discontinued treatment [35]. Despite strong preclinical activity that seemed mechanistically sound, these results did not translate into clinical benefit with DSF therapy. Compared with standard care, the overall DSF effects were harmful.

3.4. Inflammation, Sepsis and COVID-19

Disulfiram covalently modifies Cys191 of human gasdermin D (Cys192 in mouse), blocking pore formation at nanomolar concentrations and preventing interleukin-1β release and pyroptotic cell death without inhibiting upstream inflammasome assembly [5]. This represents an anti-inflammatory rather than antimicrobial mechanism, and it was the stated rationale for advancing DSF into COVID-19 trials [39]. No efficacy results from those trials have been published, so preclinical evidence remains the only support for the indication.

The same mechanism is directly relevant to interpreting any symptom-based endpoint in an infectious indication because it predicts symptomatic improvement by suppressing inflammatory signaling, irrespective of any effect on pathogen burden.

3.5. Antiviral and Antibacterial Potential

Evidence remains preclinical. Dithiocarbamates show activity against non-replicating *Mycobacterium tuberculosis* in vitro [40], and DSF inhibits SARS-CoV and MERS-CoV papain-like proteases in enzymatic assays [41].

It entered COVID-19 trials based on pyroptosis blockade [5,39], but no published efficacy results are available. Both effects likely involve covalent modification of catalytic cysteine residues rather than indication-specific pharmacology, and neither has progressed beyond initial in vitro and enzymatic experiments in their respective fields.

3.6. HIV Latency Reversal

A phase 2 dose-escalation study using DSF as a latency-reversing agent demonstrated an effect on the latent reservoir, a pharmacodynamic rather than clinical endpoint. It has not progressed to efficacy trials [38].

3.7. Obesity and Metabolic Syndrome

In mice consuming an obesogenic diet, DSF decreased feeding efficiency, reduced body weight, boosted energy expenditure, enhanced glucose tolerance and insulin sensitivity, and lessened metabolic dysfunction across multiple organs [42]. Combined transcriptomic and proteomic studies of mouse and rat livers revealed shared signatures in lipid and energy metabolism, redox balance, and detoxification, and showed that DSF strongly activates autophagy in cell culture [27]. Inhibition of fructose-1,6-bisphosphatase and α-glucosidase has also been suggested as additional mechanisms contributing to glycemic regulation [46].

Human studies on this approach are registered but not yet published, so current evidence is limited to animal and mechanistic research.

3.8. Cocaine Use Disorder

Investigated off-label for dopamine beta-hydroxylase inhibition rather than aldehyde dehydrogenase inhibition [1]; randomized trials combining DSF with behavioral therapy showed reduced cocaine use in some groups, but findings have been inconsistent across studies [37].

3.9. Dermatology

Chelation therapy for nickel dermatitis, based on a small double-blind study, serves as a reference for comparator hepatotoxicity estimates later cited in the safety literature [43].

3.10. Overview Across DSF Repurposing Indications

Data reported in Table 2 reveals two patterns.

First, the strong, mechanistically detailed preclinical activity is followed by small or inconsistent controlled results. In the one adequately powered comparison against standard care, no DSF benefit was observed, but considerable toxicity was noted [34,35].

Second, the same chemistry that interacts with NPL4, the proteasome, DNMT1, gasdermin D, and viral proteases also affects hepatic, neuronal, and mitochondrial targets. As a result, DSF dose alone did not allow researchers to distinguish therapeutic effects from toxicities [28]. This pattern offers insights into the Lyme question.

Apart from alcohol use disorder, DSF has repeatedly shown strong preclinical activity but inconsistent or negative controlled results. The only randomized trial directly comparing it to standard care in another indication found no benefit and significantly higher toxicity [35]. Accordingly, the prior probability of clinical benefit in a new indication should be set lower.

4. Disulfiram Toxicology and Safety

Disulfiram is administered orally; it is well absorbed and extensively metabolized in the liver into diethyldithiocarbamate and then into carbon disulfide. It irreversibly inhibits aldehyde dehydrogenase, so enzyme activity returns only with new enzyme synthesis. As a result, its effects can last up to 14 days after the last dose [1,6]. Contraindications established in the class literature include severe or unstable cardiac disease, hepatic impairment of any degree, psychosis, severe renal impairment, pregnancy, and inability to understand or comply with ethanol avoidance [1,30,33]. Continuous monitoring typically includes baseline and periodic liver function testing, with the first weeks of treatment representing the highest-risk window [47,48].

The clinical toxicity aspects in Table 3 map to identifiable molecular targets, and separating them is useful because each predicts a different monitoring strategy. Moreover, each target recorded in Table 3 is also available at therapeutic exposure, not only in DSF overdose.

4.1. Pharmacotoxicological Considerations of DSF Oral Administration

Most harms do not stem from DSF itself; instead, they are driven by DSF metabolites involved in the ethanol reaction, inhibition of dopamine beta-hydroxylase, neuropathy, and interaction risks [6,7,9,10]. As a result, oral dosage only loosely predicts toxicity, and hepatic impairment—which affects metabolite ratios—is an absolute rather than relative contraindication [30,31].

Table 3. DSF toxicology: mechanisms, frequency, course and molecular targets.

Toxic Event	Mechanism	Frequency and Timing	Course and Management	Reference
DSF-ethanol reaction	Acetaldehyde accumulation after ALDH inhibition: flushing, throbbing headache, nausea, vomiting, tachycardia, hypotension, dyspnea	On any ethanol exposure during treatment and for up to 2 weeks after stopping; severity is dose-related in both drug and ethanol	Ranges from unpleasant to life-threatening with arrhythmia, myocardial infarction, cardiovascular collapse, seizures and death; deaths are now rare because lower doses are used and cardiac patients excluded	[1,14,47]
Idiosyncratic hepatotoxicity	Immuno-allergic injury attributed to accumulation of diethyldithiocarbamate and carbon disulfide with oxidative stress, mitochondrial dysfunction and hepatocellular necrosis	Hepatitis is estimated at 1 in 25,000 to 1 in 30,000 patients treated per year; most cases arise within the first 2 to 12 weeks; can occur at any point regardless of baseline liver function	Ranges from asymptomatic transaminase elevation to fulminant hepatic failure; fatal cases reported despite discontinuation; usually reverses on withdrawal; positive rechallenge documented	[29,31,47,48]
Peripheral neuropathy	Distal axonal sensorimotor degeneration attributed to carbon disulfide and dithiocarbamate metabolites	Approximately 1 case per 10,000 patients treated per year; dose-related, more frequently above 250–500 mg/day; onset typically after weeks to months	Improves over weeks to months after withdrawal; incomplete recovery reported in a minority	[2,49]
Central neurotoxicity	Dopamine beta-hydroxylase inhibition and metabolite accumulation	Rare; encephalopathy, seizures and optic neuritis described in case reports; dose-dependent	Resolves in discontinuation in most reported cases	[1,30,50]
Psychiatric reactions	Altered catecholamine metabolism; dose-dependent	Rare at licensed doses: psychosis, confusional states, mutism, memory impairment, stupor; more frequent at higher doses	Resolve within days to weeks of withdrawal, occasionally requiring antipsychotic treatment	[1,30,51]
Cardiac events	Hemodynamic stress of the ethanol reaction; direct effects less well defined	Heart failure and death reported in patients with significant coronary disease or pre-existing heart failure	Basis for excluding significant cardiac disease before initiation	[1,30]
Dermatological	Hypersensitivity; also contact sensitization from the dithiocarbamate structure	Rash, pruritus and exfoliative dermatitis reported rarely	Withdrawal; the same chemistry underlies its historical use in nickel dermatitis	[30,43]
Drug interactions	Inhibition of hepatic cytochrome P450-mediated metabolism	Clinically important with warfarin, phenytoin, isoniazid, metronidazole, benzodiazepines and theophylline	Requires documented interaction review and dose adjustment before initiation	[2,16,30]

Molecular Target	Species Responsible	Toxic Event	Mechanism	DSF Dose Relationship	References
ALDH1A1/ALDH2	MeDDC sulfoxide and sulfone	DSF-ethanol reaction: flushing, headache, vomiting, tachycardia, hypotension; rarely arrhythmia, myocardial infarction, collapse and death	Irreversible carbamoylation of the active-site cysteine; acetaldehyde accumulates on any ethanol exposure and remains elevated for up to 14 days after the last dose	Severity scales with both drug and ethanol dose	[6,9,47]
Dopamine beta-hydroxylase	DDC	Psychosis, confusional states, mutism, stupor, memory impairment; contributes to orthostatic hypotension	Copper-dependent enzyme inhibited by chelation; dopamine accumulates, and noradrenaline synthesis falls	Clearly dose-dependent; rare at 250 mg/day or below	[1,51]
Axonal neurofilament proteins	Carbon disulfide and dithiocarbamate metabolites	Distal symmetrical sensorimotor axonal neuropathy; encephalopathy; optic neuritis	Cross-linking of neurofilament proteins producing distal axonal degeneration, the same lesion as industrial carbon disulfide exposure	Approximately 1 case per 10,000 treated per year; markedly more frequent at above 250–500 mg/day	[8,49,50]
Hepatic cytochrome P450 enzymes	DSF and DDC	Interaction toxicity: warfarin bleeding, phenytoin toxicity, and accumulation of isoniazid, metronidazole, benzodiazepines, and theophylline	Mechanism-based inhibition by thiocarbamate sulfoxides that are themselves cytochrome P450 products	Present at all therapeutic doses	[7,10]
Mitochondrial matrix proteome	DSF and metabolites	Hepatocellular injury; plausible contributor to fatigue and to neurotoxicity	Induction of protein insolubility specifically within the mitochondrial matrix with impaired oxidative metabolism, independent of ALDH inhibition	Concentration-dependent in vitro	[28]
Hepatic thiol pool and glutathione	DSF and DDC	Idiosyncratic hepatitis, from asymptomatic transaminase elevation to fulminant hepatic failure; fatal cases despite withdrawal; positive rechallenge documented	Metabolite-driven oxidative and mitochondrial stress with an immune-allergic component; entirely hepatic metabolism concentrates exposure	1 in 25,000 to 30,000 per treated year; most cases within 2–12 weeks; independent of baseline liver function	[29,31,48]

Table 3. *Cont.*

Molecular Target	Species Responsible	Toxic Event	Mechanism	DSF Dose Relationship	References
Copper and zinc homeostasis	DDC and CuET	Redox stress in hepatocytes and neurons, underpinning several of the toxicities above	Chelation forms lipophilic bis(DDC)-metal complexes that redistribute intracellular copper and catalyze Fenton-type ROS generation	Modulated by dietary and tissue copper status	[3,18]
p97/NPL4 segregase and 20S proteasome	CuET	Proteotoxic stress in non-malignant tissue; contributes to the excess of grade 3 or higher events when copper is co-administered	NPL4 immobilization and inhibition of chymotrypsin-like proteasome activity are not restricted to tumor cells	Dose-limiting toxicity at 1000 mg/day in glioblastoma trials	[3,17,35,36]
Gasdermin D (Cys191)	DSF	Theoretical immunosuppression: blunted IL-1beta release and pyroptosis with impaired innate control of intracellular pathogens	Covalent modification blocks pore formation at nanomolar concentrations, the same mechanism sought therapeutically in sepsis	Active at low exposure	[5]
PD-L1	DSF/Cu complex	Immunosuppression within tumor tissue, opposing the intended immune mechanisms	Stabilization of PD-L1 expression	Copper-dependent	[26]
Dithiocarbamate contact sensitization	DSF	Rash, pruritus and exfoliative dermatitis	Hapten formation by the dithiocarbamate group, the same chemistry exploited historically in nickel dermatitis	Idiosyncratic	[30,43]

Two targets are both therapeutic and potentially toxic. Inhibiting NPL4 and the proteasomes serves as an anticancer mechanism and contributes to the higher incidence of grade 3 or higher adverse events in the glioblastoma trial [3,35], whereas gasdermin D blockade has anti-inflammatory benefits but also raises a theoretical risk of infection [5]. The molecule's structure, rather than its pharmacokinetics, prevents dose-based separation of these effects.

Monitoring strategies vary by target: hepatic injury requires scheduled biochemistry tests during the first 2 to 12 weeks [29,48]; neuropathy involves clinical exams after weeks to months and can largely be avoided by keeping doses below 250–500 mg/day [49]; and cytochrome P450 interactions require a one-time prescription review before starting treatment, rather than ongoing surveillance.

4.2. Pharmacotoxicological Considerations on DSF Use in Lyme Disease

DSF has been clinically used for over seventy years, so its toxicology, summarized in Table 3, is much better understood than its anti-borrelia effects [1,30].

The context matters, because the available studies of DSF benefit in Lyme disease involved a small number of participants; thus, the rare side effects of DSF were not detectable, while the mechanisms of common toxicities are identical, regardless of purpose.

Two consequences of this profile shape how Lyme borreliosis data should be interpreted.

First, the rare and most serious DSF-induced events (fulminant hepatic failure, psychosis, optic neuritis, and cardiac death) occur at frequencies of 1 in 10,000 to 1 in 30,000 treated patients per year [30,47–49]. Therefore, the available human studies comprising 93 exposed patients were unable to detect them. Hence, their absence from the Lyme reports is uninformative rather than reassuring.

Second, current hepatology guidance does not allow DSF administration in individuals with liver disease of any degree because it is entirely hepatically metabolized and hepatotoxic [30,31]; it is a stricter constraint than most Lyme prescribing practice has applied [11].

5. Disulfiram in Lyme Borreliosis

This section systematically reviews available data on DSF in Lyme borreliosis. First, it narratively describes Lyme disease, its burden, and the limitations of current therapy,

followed by the hypotheses and the review question. This section then continues with systematic search methods, retrieved evidence, and its appraisal.

5.1. Lyme Borreliosis: Definition and Nomenclature

Lyme borreliosis (Lyme disease) is a multisystem infection transmitted by ticks of the genus *Ixodes*, mainly *I. ricinus* in Europe, *I. persulcatus* in Asia, and *I. scapularis* and *I. pacificus* in North America. It is caused by spirochetes from the *Borrelia burgdorferi sensu lato* complex. This review uses the terms "Lyme borreliosis" and "Lyme disease" interchangeably, with the former common in Europe and the latter in North America.

Diagnosis relies on clinical signs supported by laboratory tests when necessary. The EUCALB consortium's European case definitions list specific clinical manifestations and the laboratory criteria deemed essential or supportive for each. The North American guidelines come from the IDSA/AAN/ACR. Clinicians diagnose erythema migrans clinically without serology, while other symptoms require laboratory confirmation—typically two-tier serology, with culture or PCR used for confirmation rather than initial screening.

5.1.1. Stages of Recognized Clinical Manifestations

- Early localized—erythema migrans, a spreading red or bluish-red lesion at least 5 cm in diameter, with or without central clearing [52].
- Early disseminated—multiple erythema migrans spots, borrelial lymphocytoma, early neuroborreliosis (most often meningoradiculoneuritis, Garin-Bujadoux-Bannwarth syndrome in Europe), Lyme carditis with conduction issues, and eye involvement [52,53].
- Late—acrodermatitis chronica atrophicans, Lyme arthritis, and advanced neuroborreliosis including encephalomyelitis [52,54].
- Post-treatment persistent symptoms—fatigue, pain, and neurocognitive problems lasting over six months after treatment. This category is symptom-based, not an established infectious diagnosis, and is called post-treatment Lyme disease syndrome in research contexts [55,56].

5.1.2. Genospecies Correlate with Clinical Presentation

B. afzelii mainly causes skin issues, *B. garinii* is linked to neuroborreliosis, and *B. burgdorferi sensu stricto* is associated with arthritis, a link first identified in European clinical isolates [57] and confirmed by later studies [54]. Two clarifications remain consistent: relapsing-fever group borrelioses, including *B. miyamotoi* infection, are separate diseases from Lyme borreliosis [58]; co-infections with *Babesia* and *Bartonella* species are distinct from Lyme disease manifestations [11,54].

5.2. Global Burden and Public-Health Emergency of Lyme Disease

Lyme borreliosis is the most common vector-borne disease reported in the Northern Hemisphere, and its impact is increasing. A systematic review and meta-analysis estimated global exposure at 14.5% (95% CI 12.8–16.3%), with the highest regional rates in Central Europe (20.7%), Eastern Asia (15.9%), and Western Europe. Seropositivity was more common in individuals over 50, men, rural residents, and those bitten by ticks (18.8%) [59].

In Europe, a review of studies from 2005 to 2020 found national seroprevalence from 2.7% in Norway to 20% in Finland. Among general populations tested with a two-tier approach, seroprevalence was higher in Western (13.6%) and Eastern Europe (11.1%) than in Northern (4.2%) or Southern Europe (3.9%). It was significantly higher in tick-exposed groups (40.6%) compared to the general population (3.9%) [60]. In the U.S., insurance-claims data estimated about 476,000 diagnoses annually from 2010 to 2018, approximately ten times higher than surveillance reports [61]. European surveillance is fragmented,

lacking a unified EU system and consistent case definitions, which leads to underestimation of true incidence [52,60].

Several factors contribute to this growing problem. Climate warming is expanding the range, density, and seasonal activity of *Ixodes ricinus* to northern areas and higher altitudes, broadening transmission zones [62]. Diagnosis remains indirect and imperfect: serology cannot differentiate active from past infections and is less sensitive early on, while culture and PCR are confirmatory, not screening tools [52,63]. Currently, no licensed human vaccine exists; though an OspA-based candidate is in late-stage trials, it is not yet available [64]. Consequently, prevention, diagnosis, and treatment face significant challenges.

5.3. Current Therapeutic Protocol in Lyme Disease: Efficacy and Adverse Effects

Lyme disease treatment varies by stage and involves antibiotics. Typical regimens for erythema migrans include oral doxycycline, amoxicillin, or cefuroxime axetil, usually for 10–14 days. Neuroborreliosis is treated with doxycycline or intravenous ceftriaxone, with oral doxycycline considered equivalent to intravenous therapy in early European cases. Lyme arthritis is managed with oral antibiotics, escalating to parenteral treatment if refractory. High-grade carditis requires parenteral therapy. The protocol shows good results based on its endpoints: in randomized trials of erythema migrans, about 90–95% of evaluable patients achieved satisfactory outcomes, with no significant difference between doxycycline and cefuroxime axetil. Shorter courses, such as 10 days versus 15, or 7 versus 14 days, have proved similarly effective in European adults, with low rates of treatment failure or late complications. Serious treatment failure and progression are rare after appropriate therapy.

5.4. Persistent Symptoms of Lyme Disease After Treatment and the Unmet Need

Lyme borreliosis is the most common vector-borne disease in the Northern Hemisphere, with *B. burgdorferi sensu lato* detected in *Ixodes ricinus* across 23 European countries [54,65]. After recommended antibiotic therapy, a subset of patients reported persistent or relapsing fatigue, pain, and neurocognitive symptoms lasting beyond six months. The research literature describes these symptoms as post-treatment Lyme disease syndrome (PTLDS) and elsewhere as chronic or persistent Lyme disease [55]. Prospective cohort studies place the burden of this residual symptom load on firmer footing than clinic-based series allow. In a Dutch observational cohort, the prevalence of persistent symptoms after treatment was quantified against matched controls [66], and in a prospective cohort of patients with ideally treated early Lyme disease, a measurable proportion still met criteria for post-treatment Lyme disease at follow-up [67].

Proposed mechanisms of persistence include immune dysregulation, residual bacterial antigen, microbiome alteration, altered neural circuitry, and the survival of antibiotic-tolerant *Borrelia burgdorferi* persister forms that tolerate doxycycline, amoxicillin, and ceftriaxone in vitro and in animal models [68–71]. The persister hypothesis has driven repurposing screens for active agents against both replicating and non-replicating subpopulations [69].

The unmet need is therefore specific, not general. Standard antibiotics reliably clear objective early disease [72–74] but do not eliminate antibiotic-tolerant subpopulations in vitro or in animal models [68,70,71], and extending or repeating conventional therapy in symptomatic patients has failed in controlled trials while adding harm [75,76]. What is missing is an agent active against non-replicating forms, tolerable enough for outpatient use, and testable against an objective endpoint. Screening approved compounds for anti-persister activity is one response to that gap [4,69], and DSF emerged from precisely such a screen [4].

5.5. Hypotheses Advanced for DSF Benefits in Lyme Borreliosis

Based on the literature, five distinct hypotheses can be formulated [2,4,11]. These are explicitly outlined here because the clinical studies discussed later were not designed to directly test any of them [11,16,44,45]:

5.5.1. H1—Persister Killing

DSF targets antibiotic-tolerant stationary-phase and round-body forms that survive standard therapy, potentially addressing the persistent infection reservoir [4,69].

5.5.2. H2—Metal Cofactor Deprivation

By competing for manganese and zinc, DSF metabolites disrupt the Borrelia metal transporter A pathway and the Mn- and Zn-dependent virulence systems. Because *B. burgdorferi* is not iron-dependent, targeting Mn/Zn is a promising selective strategy [11–13].

5.5.3. H3—Redox and Thiol Disruption

The spirochete depends on coenzyme A disulfide reductase and related thiol systems for oxidative stress defense, which are plausible targets for electrophilic disulfides [11,15].

5.5.4. H4—Biofilm Penetration

DSF and its CS_2 metabolite are small and lipophilic enough to infiltrate biofilm-like structures that block conventional antibiotics, as demonstrated in vitro and in post-mortem human tissues [11,77,78].

5.5.5. H5—Immunomodulation Rather than Eradication

Mechanisms such as gasdermin D blockade, lowered inflammatory markers, and altered antibody and lymphocyte profiles might reduce symptoms without eliminating bacteria, meaning clinical improvement could occur despite failure to fully eradicate the pathogen [5,79].

5.5.6. Observations

H1–H4 suggest microbiological eradication, whereas H5 implies symptom relief without it. No reviewed human studies included microbiological endpoints [11,16,44,45]; thus, these hypotheses remain empirically indistinguishable in patients, as discussed later.

5.6. Other Candidate Agents Evaluated for Lyme Borreliosis

Disulfiram is one of several compounds proposed for Lyme disease since 2014; data summarized in Table 4 are narrative and contextual, not part of the systematic search.

5.7. Rationale of the Study Design and Systematic Review Question

In 2016, a screen of 4366 compounds identified DSF as the single most active approved molecule against *B. burgdorferi* [4]. Three favorable case narratives published in 2019 [44] were followed by rapid, patient-driven off-label prescribing. Because DSF carries well-characterized hepatic, neurological, psychiatric, and alcohol-interaction hazards [2,14,29,32], the mismatch between uptake and evidence warrants formal appraisal.

This systematic appraisal addresses the following question: in patients with Lyme borreliosis or persistent post-treatment symptoms, what evidence reports that DSF improves clinical outcomes, and what evidence shows its harmful effects?

Table 4. Other evaluated agents against *B. burgdorferi* since 2014.

Agent/ Strategy	Highest Level of Evidence	Key Quantitative Finding	Status and Limitation	Reference
Hygromycin A	In vitro plus mouse infection model	Selective spirochaete inhibitor from Streptomyces hygroscopicus; taken up via a spirochaete-specific nucleoside transporter; concentrations needed to kill gut commensals were 17- to >1000-fold higher than for B. burgdorferi; cleared infection in mice, including by oral bait	Most promising novel agent; microbiome-sparing by design; no human trial published	[80]
Azlocillin (+/- cefotaxime)	In vitro plus C3H/HeN mouse model	Completely killed late-log and 7–10-day stationary-phase *B. burgdorferi* and doxycycline-tolerant persisters; combination with cefotaxime effective against doxycycline-tolerant organisms; efficacy confirmed in mice	Parenteral ureidopenicillin; no human trial in Lyme disease	[81]
Nitroxoline-based combinations	In vitro, persister-enriched stationary phase with subculture	Nitroxoline + cefuroxime, nitroxoline + clarithromycin and the triple combination matched the daptomycin-based positive control, eradicating stationary-phase organisms and preventing regrowth	Outperformed every DSF-containing regimen in the same laboratory; not yet tested in animals or humans	[82,83]
Daptomycin + cefoperazone + doxycycline	In vitro, with subculture; mouse model	The reference persister-eradicating combination in vitro; combination regimens eradicated persistent infection in mice where all monotherapies, DSF included, failed	Requires parenteral daptomycin; not evaluated in a controlled human trial	[84,85]
Dapsone combination therapy	Uncontrolled clinical series	Retrospective series of 200 patients reported reduction in eight major symptom scores; double-dose protocols reported remission for a year or longer in approximately half of treated patients; 46 of 200 discontinued for adverse reactions	Same evidential weaknesses as the DSF series: single practice, no control, no blinding; dapsone carries hemolysis, methemoglobinemia, agranulocytosis and neuropathy risk	[86–88]
OspA vaccine (VLA15)	Phase 3	OspA-based candidate in late-phase efficacy trials; no licensed human Lyme vaccine currently available	Prevention rather than treatment; efficacy data not yet published	[64]
Anti-OspA monoclonal antibody (pre-exposure prophylaxis)	Phase 1 completed; phase 2/3 planned	Long-acting human monoclonal antibody targeting OspA, intended to neutralize Borrelia in the tick midgut before transmission via a single seasonal injection	Prevention rather than treatment; current information is largely from company communications rather than peer-reviewed trial reports	[89]

5.8. Methods

This review reports its systematic component in accordance with the PRISMA 2020 statement [90], with the search reported according to the PRISMA-S extension [91]; the completed 27-item checklist and full search strategies are provided in Supplementary Table S4. We did not register or deposit a protocol for this review, prospectively or retrospectively.

The eligibility criteria, search strategy, extraction fields, and risk-of-bias instruments reported in Sections 5.9–5.11 were specified before the searches were executed, but that specification exists only within this manuscript and cannot be independently verified against a time-stamped record.

Because heterogeneity in design, population definition, dosing, and outcome instruments precluded statistical pooling, the synthesis is narrative-quantitative rather than a meta-analysis; this decision was taken before the data were assembled and is not a post hoc response to the retrieved data. We report the synthesis without meta-analysis following the SWiM guideline [92].

5.9. Eligibility Criteria

- Population: *B. burgdorferi sensu lato* (via in vitro, animal models), or humans diagnosed with Lyme borreliosis, PTLDS, or chronic/persistent Lyme disease.
- Intervention: DSF or its metabolites, used alone or combined, in any dose or duration.
- Comparator: any, including no comparator (uncontrolled studies are included due to limited evidence).
- Outcomes: microbiological eradication, symptom severity, quality of life, functional status, remission duration, and adverse events.
- Designs: high-throughput and targeted in vitro tests, animal studies, case reports and series, patient surveys, both controlled and uncontrolled trials.
- Exclusions: narrative reviews, editorials, commentaries lacking primary data, non-*Borrelia* treatments, and reports without specific DSF-related outcomes.

5.10. Information Sources and Search

We distinguish three categories of sources:

5.10.1. Bibliographic Databases

PubMed (MEDLINE), Europe PMC and the Cochrane Library, including CENTRAL. These carry out the search and determine the yield. PubMed and Europe PMC are not interchangeable: PubMed indexes citations and searches title, abstract, and MeSH fields, whereas Europe PMC additionally searches deposited full text and indexes preprints, so it retrieves occurrences that PubMed cannot reach; in this review, Europe PMC returned every PubMed record and 159 further records.

5.10.2. Preprint Servers and Trial Registries

BioRxiv, medRxiv, and ClinicalTrials.gov were searched to capture unpublished and in-progress work.

5.10.3. Supplementary Hand-Searching

Frontiers and MDPI are publisher platforms, not indexes; we searched them only as a redundancy check because several key reports in this literature appear in their journals, and they contributed no unique records. Backward citation chasing was performed on all included reports and on the 2023 mechanistic review by Lanz et al. [2].

We searched all sources from inception to the execution date recorded for each in Supplementary Table S1. No date, language, or publication-status limits were applied. Every source was searched twice, on two separate occasions, and the two yields were compared source by source before the records were pooled; where the counts differed, the search was repeated and the discrepancy resolved before the record was accepted into the library.

No source therefore rests on a single execution, and the per-source counts reported in Supplementary Table S1 are reconciled. Preprints were eligible but were replaced by the peer-reviewed version where one existed. Full, executable search strings for every source, with the date each was run and the number of records returned, are given in Supplementary Table S1, while the concepts underlying them are summarized in Table 5.

Table 5. Search concepts and terms.

Concept	Terms Combined with OR	Combined with	Records Yielded (References)
Intervention	DSF; tetraethylthiuram disulfide; Antabuse; diethyldithiocarbamate; DDC	AND	[2,4,11,14,16,44,45,79,83,84]
Condition	Lyme; borreliosis; *Borrelia burgdorferi*; PTLDS; post-treatment Lyme disease; chronic Lyme disease	AND	[11,16,44,45,55,85]
Outcome/design	persister; stationary phase; drug repurposing; clinical trial; case series; adverse events; toxicity	—	[4,6,29,32,68–71,79,83,84]
Reporting framework	PRISMA 2020 checklist and flow diagram	—	[80]

5.11. Data Extraction and Risk of Bias

Every stage of the systematic component was carried out twice by a single researcher. No second screener was available, so the review does not claim independent duplicate screening at any point, and single screening is known to miss a small but non-trivial proportion of eligible records relative to conventional double screening [93]. What was done instead was to perform each of the three error-prone stages twice rather than once, on separate occasions, so that no reported figure rests on a single reading.

5.11.1. Data Searching, Extracting and Reporting

First, the search: every database, registry and preprint server listed in Supplementary Table S1 was executed on two separate occasions and the yields compared source by source, with any discrepancy resolved by re-running that source before its records were accepted.

Second, the selection: title and abstract screening and full-text assessment were each performed twice against the same written eligibility criteria, the second occasion serving as a verification pass, and a record was classified only once the two passes agreed; the classification entered into the PRISMA flow is therefore the settled one rather than a first impression.

Third, the reporting of results: data were extracted against a piloted, pre-specified form (Supplementary Table S2), and the extraction and the transcription of those values into the tables and text of this review were likewise carried out twice. We also checked every numerical value—denominators, event counts, percentages, doses, follow-up durations, and confidence intervals—against the source publication rather than the first transcription alone, because an error copied forward from a first reading would otherwise survive a second. This step is reported explicitly because transcription error, rather than judgment, is the commonest way a single-reviewer synthesis misstates what its sources report.

No agreement statistics are reported. Cohen's κ measures agreement between independent raters, and because a single author conducted this process, no inter-rater value exists. A figure computed from two passes by the same researcher would measure that person's consistency, not the reliability of the selection, and we do not present one. Extracted fields comprised study identity, design, setting and country, organism strain, growth phase and assay for preclinical work, patient population, dosing regimen in mg/day and mg/kg/day, treatment duration, length of follow-up, the population analyzed and the population enrolled, missing data and attrition, efficacy outcomes with the instrument and threshold used, adverse events with frequency and reversibility, and funding and conflict-of-interest statements.

5.11.2. Risk of Bias Assessment

Risk of bias was assessed using design-appropriate, validated instruments rather than ad hoc domains: ROBINS-I for non-randomized human studies [94], RoB 2 for the randomized pilot trial [95], and SYRCLE's risk-of-bias tool for animal experiments [96]. Because no validated instrument exists for in vitro antimicrobial work, we used a pre-specified domain set covering strain provenance, inoculum and growth phase, formulation and solvent control, concentration range, viability assay validity, blinding of outcome assessment, and independent replication (Supplementary Table S3).

Three of the four human studies are rated at critical risk of bias on ROBINS-I. Current guidance holds that studies at critical risk of bias should not be included in a synthesis alongside studies at lower risk, or should be presented separately from them [94,97]. We have retained them and state the reasoning explicitly rather than leaving it implicit. Excluding them would reduce the human evidence base to a single nine-participant pilot trial and would defeat the purpose of the review, which is to characterize the evidence driving off-label prescribing, not only the evidence that meets a methodological threshold. They are therefore reported as a separate stratum, their domain ratings are given in full in Supplementary Table S3, and no conclusion in this review rests on them beyond the statement that a benefit signal exists and cannot be distinguished from bias.

Certainty of evidence was rated with GRADE [98], adapted for a body of evidence dominated by non-comparative designs in accordance with the guidance on integrating ROBINS-I judgments into certainty ratings [99]: each outcome began at the certainty appropriate to its design and was downgraded explicitly for risk of bias, inconsistency, indirectness, imprecision and publication bias.

5.12. Pathogen Scope: Genospecies and Related Borrelioses

We intentionally defined eligibility at the broad *sensu lato* complex level to include studies of any genospecies. However, we identified only *B. burgdorferi sensu stricto*. We excluded relapsing-fever borrelioses because they are considered separate diseases. This mismatch between the criteria and the available evidence is reflected as a species-indirectness downgrade in the certainty assessment, which we discuss further under applicability (Table 6).

Table 6. Pathogens involved in Lyme borreliosis.

Pathogen	Distribution	Characteristic Clinical Association	DSF Evidence Base	Ref
B. burgdorferi sensu stricto	North America; uncommon in Europe	Lyme arthritis; disseminated disease	The sole organism in each in vitro and animal study, almost always strain B31	[4,54,79,83,84]
B. afzelii	Europe and Asia; most widely distributed genospecies in Romania	Cutaneous disease, including acrodermatitis chronica atrophicans	No data	[54,65,93,94]
B. garinii (and *B. bavariensis*)	Europe and Asia; second most frequent in Romania	Neuroborreliosis		
B. spielmanii, *B. lusitaniae*, *B. valaisiana*, *B. bissettii*	Europe, focal	Occasional or contested human pathogenicity		[65,100]
B. mayonii	Upper Midwestern United States, rare	Lyme disease with high spirochetemia		[54]
Relapsing-fever group: *B. miyamotoi*, *B. hermsii*, *B. recurrentis*	Worldwide; *B. miyamotoi* present in Romanian *I. ricinus*	Relapsing fever—a distinct disease, NOT Lyme borreliosis	Out of scope; three patients in [11] carried a tick-borne relapsing fever diagnosis as a separate co-infection	[58]
Babesia microti/*B. duncani*, *Bartonella henselae*	Co-transmitted	Co-infections, not borrelioses	Reported as clinical co-infection subgroups	[11]

Clinical stage—early localized, early disseminated, and late disease—is a separate axis from genospecies and is reported inconsistently across the included human studies; all human participants were in the persistent post-treatment category. We synthesized findings by evidence tier: mechanism, in vitro, in vivo, uncontrolled human, controlled human, and safety. Quantitative results are reported as originally published; we did not re-analyze or impute data, with one exception noted here.

Where a study reported a bare proportion without a measure of precision, we computed a 95% confidence interval from the published numerator and denominator using the Wilson score method [101], which is preferred to the normal-approximation interval for small denominators and for proportions at or near zero or one [102]. These intervals describe the precision of each individual study and are not pooled. Meta-analysis was not attempted.

5.13. Studies Selection

We exported records from all sources into a single reference library, de-duplicated automatically by DOI and then by normalized title plus first-author surname plus publication year, and manually inspected each remaining candidate duplicate pair before removal. The searches returned 199 records: 16 from PubMed, 175 from Europe PMC, 7 from bioRxiv, 0 from medRxiv, 1 from ClinicalTrials.gov and 0 from the Cochrane Library. Seventeen were duplicates—all 16 PubMed records were also retrieved by Europe PMC, as was one of the seven bioRxiv records—and one further record was the preprint of the present review, which was removed as the index publication (Figure 2).

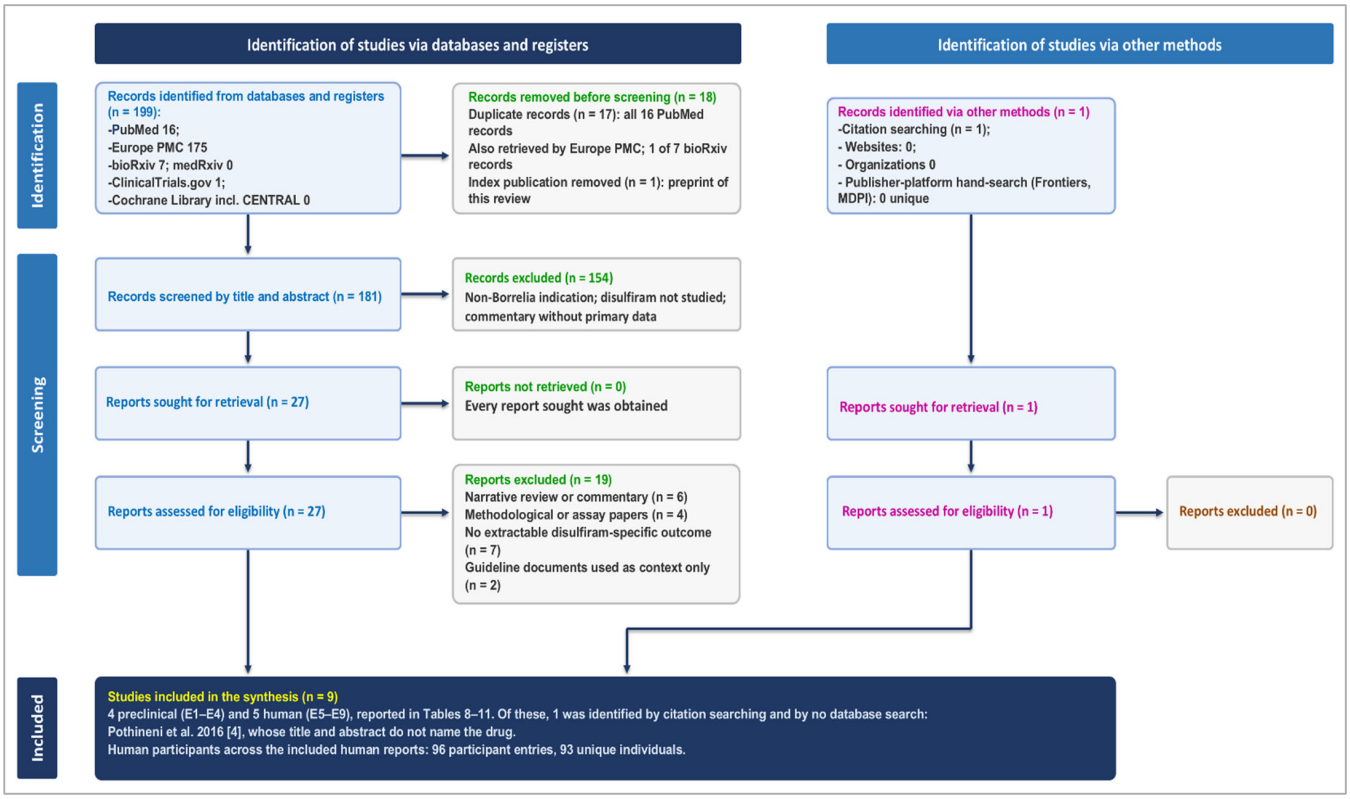


Figure 2. PRISMA 2020 flow diagram of study selection for the DSF evidence in Lyme borreliosis.

This left 181 records for title and abstract screening; 154 were excluded, and 27 were taken forward for full-text assessment (Figure 2 and Table 7). The deduplicated search library, the full record list at each stage, the screening decisions, and the reasons for exclusion

at the full-text stage are retained by the author and are available from the corresponding author on request.

Table 7. PRISMA 2020 flow of information, included studies and principal quantitative findings.

Stage	*n*	Reason/Detail	Reference
Records identified from databases and registers	199	PubMed 16; Europe PMC 175; bioRxiv 7; medRxiv 0; ClinicalTrials.gov 1; Cochrane Library incl. CENTRAL 0. Citation chasing identified 1 further eligible report not retrieved by any database search [4]; the publisher-platform redundancy hand-search (Frontiers, MDPI) contributed no unique records.	—
Records removed before screening	18	17 duplicates (all 16 PubMed records also retrieved by Europe PMC; 1 of 7 bioRxiv records also in Europe PMC); 1 index publication (the preprint of the present review, Preprints.org PPR1339915)	—
Records screened (title/abstract)	181	—	—
Records excluded	154	Non-Borrelia indication; DSF not studied; commentary without primary data	—
Reports sought for retrieval	27	Full text of every record surviving screening was sought and obtained; none was unretrievable	—
Reports assessed for eligibility	27	—	—
Reports excluded	19	Narrative review or commentary ($n = 6$); methodological or assay papers ($n = 4$); no extractable DSF-specific outcome ($n = 7$); guideline documents used as context only ($n = 2$)	[2,14,85]
Studies included in synthesis	9	8 identified from databases and registers and 1 by citation searching; 4 preclinical (E1–E4) and 5 human (E5–E9); see Tables 8–11	[4,11,16,44,45,79,82,84,103]
Human participants exposed across all reports	96 entries/93 unique	3 + 67 + 16 + 9 + 1 = 96 participant entries. The three index cases of [44] are explicitly identified as Cases 18, 49 and 10 of the series in [11], so 93 individuals are unique. No other overlap could be verified from the source publications.	[11,16,44,45,103]

ID	Study	Design	*n*	Principal Result	Reference
E1	Pothineni et al. 2016	In vitro high-throughput screen	—	4366 compounds screened; 150 inhibited >90% of growth at <25 µM; 101 FDA-approved; DSF most active at 99.80% inhibition vs. doxycycline 94.14%; MBC 1.25 µM (0.371 µg/mL)	[4]
E2	Potula et al. 2020	In vitro plus C3H/HeN mouse	—	0.625–20 µM eliminated 80–94% log-phase and 78–92% stationary-phase organisms; 5 µM killed 94% of log-phase; activity declined at 50–100 µM; clearance from heart and bladder by day 28 at 75 mg/kg/day x5 intraperitoneally	[79]
E3	Alvarez-Manzo et al. 2020	In vitro, persister-enriched stationary phase	—	DSF survival rate 46.3% vs. cefuroxime 36.8%, clarithromycin 41.1%, nitroxoline 37.5%; cefuroxime+DSF 41.7% vs. cefuroxime+clarithromycin 25.9% and cefuroxime+nitroxoline 27.5%; DSF triple combinations did not prevent regrowth	[82]
E4	Alruwaili et al. 2023	Mouse model of persistent infection	—	No monotherapy including DSF eradicated persistent *B. burgdorferi*; four dual and three triple antibiotic combinations were effective	[84]
E5	Liegner 2019	Case report	3	500 mg/day for ~3 months; body weight 95–100 kg; placed doses at 4–5 mg/kg/day; Case 1 remission >3 years; Case 2 stopped at 6 weeks after syncope, well >1 year until re-exposure	[44]
E6	Trautmann et al. 2020	Patient-organization survey (France)	16	13/16 reported toxic events; 7/16 reported benefit for at least part of their symptoms	[45]
E7	Gao et al. 2020	Single-practice retrospective review	67 of 71	62/67 (92.5%) net benefit; 12/33 (36.4%) high-dose patients in enduring remission >=6 months; 0/34 low-dose	[11]

Table 7. *Cont.*

ID	Study	Design	*n*	Principal Result	Reference
E8	Kuvaldina et al. 2025	Double-blind randomized pilot, 4 vs. 8 weeks	9 analyzed	940 screened, 11 enrolled; 3/9 completed treatment; 5/9 discontinued for adverse reactions; 6/9 met fatigue MCID; terminated early	[16]
E9	Frankl et al. 2021	Case report (published as a letter)	1	Middle-aged man treated for "chronic Lyme disease" with DSF 375 mg/day plus minocycline and tinidazole, taken above prescribed doses; encephalopathy with bilateral putaminal and thalamic lesions, anion-gap acidosis, ventilator dependence; died after 2.5 months. Prior hospitalization for DSF-associated polyneuropathy 8 months earlier	[103]

Table 8. Study-level outcome data for human studies: analyzed populations, observation times, missing data, and precision.

Study	Analyzed/Enrolled	Observation Time	Missing Data and Attrition	Outcome	*n*/N (%)	95% CI
Liegner et al. 2019 [44]	3/3	Treatment ~3 months; narrative follow-up >3 years (case 1), >1 year (case 2)	None reported; selected cases	Net benefit	3/3 (100)	43.9–100
Trautmann et al. 2020 [45]	16/16 respondents; denominator of those invited not reported	Survey window July–November 2019; no systematic follow-up	Non-response rate not recoverable; self-selected respondents	Toxic events	13/16 (81.2)	57.0–93.4
				Benefit for at least part of symptoms	7/16 (43.8)	23.1–66.8
Gao et al. 2020 [11]	67/71 initiated	Treatment 6 weeks to >16 months; follow-up to July 2020; remission defined as >=6 months off anti-infectives	4/71 (5.6%) lost to follow-up; no imputation; outcomes clinician-recorded	Net benefit (all doses)	62/67 (92.5)	83.7–96.8
	33 high dose/34 low dose			Enduring remission, high dose	12/33 (36.4)	22.2–53.4
				Enduring remission, low dose	0/34 (0)	0–10.2
				Fatigue, high dose	22/33 (66.7)	49.6–80.2
				Psychiatric symptoms, high dose	16/33 (48.5)	32.5–64.8
				Peripheral neuropathy, high dose	9/33 (27.3)	15.1–44.2
Kuvaldina et al. 2025 [16]	9 analyzed/10 randomized/11 enrolled/940 screened	Primary endpoint week 10; durability assessed week 14	1 randomized participant excluded post hoc (pre-existing neurological condition); 2 withdrew from assessments; last observation carried forward	Fatigue MCID met	6/9 (66.7)	35.4–87.9
				Quality-of-life MCID met	4/9 (44.4)	18.9–73.3
				Discontinued study medication	5/9 (55.6)	26.7–81.1
				Completed full course	3/9 (33.3)	12.1–64.6
Frankl et al. 2021 [103]	1/1	Prior DSF-associated neuropathy 8 months before; 2.5 months from presentation to death	Single case report; serial imaging to day 41 reported	Fatal neurological injury	1/1 (100)	20.7–100

Proportions were reported with 95% confidence intervals calculated from the denominators published in each source; no re-analysis of individual participant data was performed. Confidence intervals are presented to convey precision, not to imply that these uncontrolled proportions estimate a treatment effect. The width of every interval in the final column is the substantive finding. No human outcome in this literature is estimated with precision sufficient to distinguish a clinically important effect from no effect: the 95% interval around the pilot trial's fatigue response (35.4–87.9%) spans almost the entire plausible range, and

the apparent contrast between dose strata in [11] rests on intervals that are wide and derive from an uncontrolled design in which assignment to dose was not random (Table 8).

Table 9. Molecular targets and mechanistic pathways proposed for DSF and its metabolites against *Borrelia burgdorferi*.

Target or Pathway	Active Species	Proposed Mechanism in *B. burgdorferi*	Evidence	Reference
Manganese transporter BmtA and the Mn-dependent program	DDC; DDC-Mn complexes	Chelation of Mn2+ deprives the spirochaete of the cofactor it uses in place of iron; BmtA-dependent Mn import is the route targeted	Indirect: BmtA-binding small molecules are borreliacidal via Mn transport inhibition, and Mn and Zn regulate virulence determinants, but DSF itself has not been shown to act through BmtA	[12,13]
Zinc-dependent regulators	DDC; DDC-Zn complexes	Competition for Zn2+ required by regulatory and structural proteins governing virulence gene expression	Indirect: Zn dependence established; no DSF-specific demonstration	[12]
Coenzyme A disulfide reductase and thiol-dependent oxidative-stress defense	DSF	Electrophilic attack on free cysteine thiols forms mixed disulfides, disabling the CoADR-centred redox system the organism uses instead of glutathione reductase	Indirect: CoADR characterized in B. burgdorferi; thiol reactivity of DSF established in other systems	[11,15]
Biofilm and aggregate matrix	Carbon disulfide; DSF	Small lipophilic species diffuse into biofilm-like aggregates that exclude conventional antibiotics; CS_2 has a molecular diameter close to CO_2	Hypothesis only: biofilm formation demonstrated in vitro and antigens persist in human tissue, but no penetration or killing study has been performed	[8,11,77,78]
Stationary-phase and round-body persisters	DSF and metabolites	Killing of non-replicating subpopulations that tolerate doxycycline, amoxicillin, and ceftriaxone	Direct but contradictory: 78–92% killing of stationary-phase organisms in one laboratory, versus a 46.3% survival rate and failure to prevent subculture regrowth in another	[4,79,83]
Whole-organism viability, replicating forms	DSF	Growth inhibition and bactericidal activity against log-phase spirochaetes	Direct: 99.80% inhibition with MBC 1.25 µM in the original screen; 94% killing of log-phase organisms at 5 µM	[4,79]
Non-monotonic exposure-response	DSF in solution	Activity declines across 50–100 µM, attributed to colloid formation, so higher exposure is predictably not more bactericidal	Direct: demonstrated in both DMSO and cyclodextrin formulations	[79]
Host gasdermin D and inflammatory signaling	DSF	Blockade of pyroptosis and IL-1beta release, and reduced inflammatory marker expression, reducing symptoms without reducing bacterial load	Direct in the host rather than the pathogen: murine infection data show reduced inflammatory markers and altered antibody and lymphocyte profiles	[5,79]
Host immune compartment	DSF	Lower IgM and IgG at day 21 and IgG2b at day 28; decreased CD19+ B cells with increased CD3+, CD3+CD4+, naive and effector memory T-cell fractions	Direct in mice; never tested as a mediator of symptom change in patients	[79]

Table 10. In vitro activity of DSF against *B. burgdorferi* across selected studies.

Parameter	Pothineni et al. 2016 (E1) [4]	Potula et al. 2020 (E2) [79]	Alvarez-Manzo et al. 2020 (E3) [82]
Growth phase tested	Log phase, screening conditions	Log and stationary phase	Stationary phase enriched with persisters
Readout	Growth inhibition, high throughput	BacTiter-Glo ATP, dark-field counting, SYBR Green I/PI	SYBR Green I/PI, subculture regrowth

Table 10. *Cont.*

Parameter	Pothineni et al. 2016 (E1) [4]	Potula et al. 2020 (E2) [79]	Alvarez-Manzo et al. 2020 (E3) [82]
Key potency value	99.80% inhibition; MBC 1.25 µM (0.371 µg/mL)	94% kill of log phase at 5 µM (1.48 µg/mL); 90–92% of stationary phase at 5–10 µM	Survival rate: 46.3% at standard 5 µg/mL exposure
Comparator performance	Doxycycline 94.14% inhibition	Doxycycline reduced log-phase viability by 90–97%	Cefuroxime 36.8%, clarithromycin 41.1%, nitroxoline 37.5%—all superior to DSF
High-concentration behavior	Not reported	Activity declines across 50–100 µM in both DMSO and cyclodextrin	Not the principal focus
Combination performance	Not tested	Not tested	Cefuroxime+DSF 41.7%, inferior to cefuroxime+clarithromycin 25.9% and cefuroxime+nitroxoline 27.5%; DSF triple combinations failed to prevent regrowth
Direction of conclusion	Strongly favorable	Favorable	Unfavorable

Table 11. Retrospective single-practice series (E7): outcomes by dose stratum.

Outcome	High Dose >= 4 mg/kg/day (n = 33)	Low Dose < 4 mg/kg/day (n = 34)	p (Chi-Square)	Ref
Symptomatic improvement	31 (93.9%)	31 (91.2%)	0.667	[11]
Enduring remission >= 6 months off anti-infectives	12 (36.4%)	0 (0%)	0.00010	[11]; index cases [44]
Fatigue	22 (66.7%)	11 (32.4%)	0.00497	[11]
Peripheral neuropathy	9 (27.3%)	1 (2.9%)	0.00520	[11]
Psychiatric symptoms	16 (48.5%)	5 (14.7%)	0.00288	[11]
Elevated liver enzymes	5 (15.2%)	5 (14.7%)	0.959	[11]; cf. [29,32]

5.14. Molecular Targets of DSF and Its Mechanistic Pathways Against Borrelia burgdorferi

The pathways listed below are included only if the referenced studies invoked them; however, none of the included studies directly tested these mechanisms in patients [11,16,44,45].

5.14.1. Thiol–Disulfide Exchange

DSF, an electrophile, forms disulfides with thiol-containing molecules, disrupting thiophilic cofactors in *B. burgdorferi* [6,11,15].

5.14.2. Metal Cofactor Competition

Formation of mixed disulfides with metals like zinc and manganese competes for cofactors crucial to spirochaete survival, aligning with the Borrelia metal transporter A (BmtA) target and the organism's Mn- and Zn-dependent virulence pathways [3,11–13].

5.14.3. Biofilm Penetration Hypothesis

Human metabolite carbon disulfide has antimicrobial properties and a molecular size similar to carbon dioxide, which easily diffuses through active biofilms; this remains unverified but has been proposed [8,11,77,78].

5.14.4. Non-Monotonic Concentration-Response

Higher DSF concentrations show reduced efficacy, likely due to colloid formation, meaning increased exposure does not necessarily enhance bactericidal effects [4,79].

5.14.5. Host Phagocyte Function

In human monocyte-derived macrophages and dendritic cells, DSF reduced opsonin-independent phagocytosis and cell migration, decreased CD45 and CD14 surface density, and induced mitochondrial oxidative stress and death in metabolically active cells [104]. The donors were patients with a range of inflammatory diseases rather than a defined Lyme population, so this study was not eligible for the systematic synthesis; it is cited here as mechanistic context for the host-directed column of Figure 3.

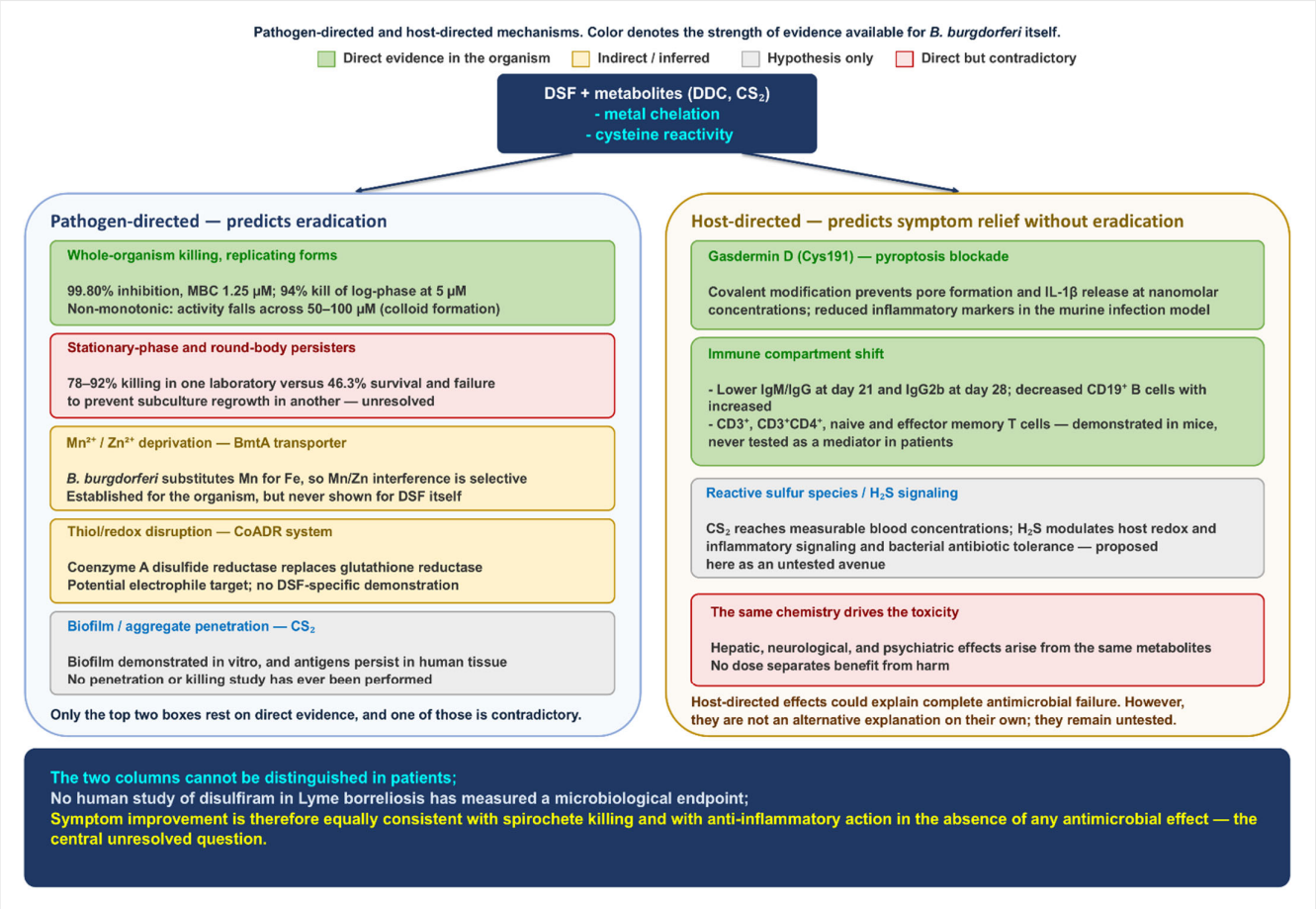


Figure 3. Proposed molecular targets of DSF in Lyme borreliosis, classified by the strength of evidence available in *B. burgdorferi*. Pathogen-directed mechanisms predict eradication; host-directed mechanisms predict symptom relief without it. Because no human study has measured a microbiological endpoint, patients cannot be distinguished by the two columns.

5.14.6. Off-Target Activity

Aside from aldehyde dehydrogenase, DSF inhibits the proteasome and affects microbial aldehyde handling, indicating poorer tolerance in flora that produces acetaldehyde [2,3,14].

5.14.7. Immunomodulation

In mice, DSF reduces inflammatory markers, lowers IgM/IgG at day 21 and IgG2b at day 28, decreases CD 19 + B cells, and increases CD 3 +, CD 3 + CD 4 +, naive, and

effector memory T cells—suggesting a possible non- antimicrobial way to modify symptoms, although this has never been tested in humans [5,79].

Table 9 summarizes the proposed molecular targets and pathways for DSF specifically against *B. burgdorferi*, highlighting those supported by direct experimental evidence in the organism versus those inferred from other systems. This distinction matters because Lyme literature often cites mechanisms proven in mammalian cells as if they were demonstrated in the spirochete.

Two key points in this table underpin the entire section's argument. First, only three rows are based on direct evidence from *B. burgdorferi*, and one is internally inconsistent between laboratories [4,79,82]. The metal-deprivation and redox hypotheses—commonly cited mechanisms supporting clinical use—are derived from the organism's biology rather than direct evidence for DSF [12,13,15]. Second, the last two rows focus on host responses rather than the pathogen. If symptom relief in patients results from gasdermin D blockade and immunomodulation, it could occur even if the antimicrobial treatment fails entirely [5,79]. Because no human study included a microbiological endpoint, patients cannot distinguish whether the effects are pathogen- or host-directed [11,16,44,45] (Figure 3).

5.15. In Vitro Evidence

All findings are presented in Table 10. The discrepancy is unreconciled and plausibly assay-driven: ATP-based viability cannot distinguish inhibitory from bactericidal effects, inoculum age and formulation differ, and only E3 applied a subculture regrowth test, the most stringent available surrogate for eradication.

5.16. In Vivo Evidence

Two animal studies present conflicting results. In the C3H/HeN model treated on days 14 and 21 after infection, DSF eliminated *B. burgdorferi* from the heart and urinary bladder by day 28, prevented cardiac histopathology, and lowered inflammatory markers [79]. Conversely, in a later mouse model designed to study persistent infection, DSF alone did not eradicate the organism, while four dual and three triple antibiotic combinations succeeded [84].

The positive study's regimen was 75 mg/kg/day for five days via intraperitoneal injection [79]. Both the original authors and later commentators recognize that this dose would be toxic in humans [11,79] and significantly higher than the 4–5 mg/kg/day used in clinical settings [11,44]. Because mice have poor oral absorption, an equivalent oral regimen was not feasible [11,79], meaning no animal study has replicated a human dosing schedule at the same exposure level. Additionally, neither study included European genospecies [54,79,84].

Consequence for translation: the only in vivo eradication signal occurs at an exposure that cannot be safely reproduced in patients [11,79], and the only study specifically designed to assess persistent infection found DSF monotherapy ineffective [84].

5.17. Human Evidence

Five human reports met eligibility and were summarized in Table 8, including their analyzed populations, observation times, missing data, and precision. The largest is an uncontrolled retrospective review of a single United States practice [11], in which 67 of 71 patients who began DSF were evaluable. Researchers stratified patients post hoc by average daily dose into a high-dose group (>=4 mg/kg/day, $n = 33$) and a low-dose group (<4 mg/kg/day, $n = 34$). Net symptomatic benefit was endorsed by 62 of 67 patients (92.5%, 95% CI 83.7–96.8), and enduring remission of at least six months off all anti-infective treatment was reached by 12 of 33 high-dose patients (36.4%, 95% CI 22.2–53.4) against

none of the 34 low-dose patients (0%, 95% CI 0–10.2). Because dose was not randomly assigned and outcomes were recorded by the treating clinicians, this contrast cannot be interpreted as a dose–response relationship (Table 11).

Co-infection subsets [11]: all 15 patients with babesiosis co-infection improved with DSF alone, with 5 (33.3%) reaching lasting remission. Among 12 patients with Bartonella henselae exposure, 10 (83.3%) reported improvements. Some individuals experienced Jarisch-Herxheimer-like reactions, prompting cautious dose adjustments. Psychiatric symptoms generally resolved within days to weeks after stopping the medication, while neuropathies improved over weeks to months, with only one case showing mild residual effects. The French patient-organization survey (E6) offers a different perspective. Initiated after a severe, long-lasting toxic reaction in a patient with late disseminated disease, it gathered responses from 16 patients: 13 experienced toxic effects, and 7 reported some symptom relief (Table 7, E6; Table 8).

The cohort included 71 initiated, 67 evaluable, 35 female, and 32 male patients, aged 17–83, treated from 6 weeks to more than 16 months; 13 patients (19.4%) received more than one course. The authors proposed 4–5 mg/kg/day for 6–12 weeks as the target for enduring remission, while noting benefit at doses as low as 0.06–2 mg/kg/day.

The authors indicated that it is too early to recommend DSF without further investigation into its toxicity mechanisms and the results of a properly designed double-blind trial (Table 11). Notably, the dose–response relationship in this study differs from the retrospective series [11]: five of six fatigue responders had received only up to six weeks of DSF, and responders appeared at both lower and higher doses [16]. Consequently, the investigators advised that future placebo-controlled trials should consider shorter treatment periods at doses under 500 mg/day [2,16].

The human evidence also contains one fatal adverse outcome, reported outside the four effectiveness reports and appraised separately in Section 5.18 and in Table 8 [103]. It is counted among the human participant entries, which brings the total to 96 entries and 93 unique individuals (Table 12).

Table 12. DSF administration in patients with Lyme disease. Results of randomized double-blind pilot study (E8, NCT03891667) and DSF side effects in patients with Lyme borreliosis.

Domain	Finding	Reference
Design	Group A: DSF 4 weeks, then placebo 4 weeks ($n = 4$); Group B: DSF 8 weeks ($n = 5$); primary endpoint at week 10, durability assessed week 14	[16]
Population	Prior documented Lyme disease with >=5 weeks of recommended antibiotics, at least partial prior response, persistent moderate or worse fatigue; extensive cardiac, hepatic, renal, neurological and psychiatric exclusions	[16]; criteria [85]
Dosing	Individualized escalation from 250 mg daily or alternate day to a maximum of 500 mg/day	[16]
Recruitment feasibility	940 screened, 11 enrolled, 10 randomized, 9 analyzed (~1%); leading screen failures were insufficient serological evidence 27.8%, pre-existing medical conditions 13.1%, geography 10.2%	[16]; cf. [56]
Tolerability	Only 3/9 completed the full medication course; 5/9 discontinued medication for adverse reactions	[16]
Serious adverse events	One 3-day hospitalization for severe abdominal pain with elevated liver enzymes at 500 mg/day, resolving on withdrawal; one emergency evaluation for possible anaphylaxis more than 10 days after stopping, judged unlikely to be related	[16]; cf. [29]
Hepatic signal	Elevated liver enzymes in 3/9, all in Group B; two had reached 500 mg/day, one only 250 mg/day; no elevations among the four Group A participants, none of whom reached 500 mg/day	[16]; cf. [29,32]
Neurotoxicity	Among six participants assessed on TNSc and NTSS-6 before and after treatment, none developed neuropathy or significant worsening	[16]; cf. [11]
Fatigue (FSS, MCID >= 0.7)	6/9 responders: 2/4 Group A, 4/5 Group B	[16]
Quality of life (Q-LES-Q, MCID >= 6.8)	4/9 responders	[16]

Table 12. *Cont.*

Domain	Finding	Reference
Multi-domain severity (PROMIS-29 SPADE)	6/9 improved	[16]
Symptom burden (GSQ-30, 50% reduction)	5/9 improved	[16]
Physical/mental function (SF-36 PCS/MCS)	3/9 and 3/9 respectively	[16]
Clinician global impression (CGI-I)	5/6 rated responders at endpoint	[16]
Durability to week 14	5/6 fatigue responders and 3/4 quality-of-life responders sustained improvement	[16]
Termination	Stopped early for end of funding, pandemic-related enrolment delay, higher-than-expected adverse events, and sufficiency of information gathered	[16]

Adverse Events of DSF in Patients with Lyme Borreliosis

Adverse Event	Best Available Frequency	Course	Reference
Fatigue	66.7% high dose; 32.4% low dose	Reversible on withdrawal	E7 [11]
Psychiatric symptoms (hypomania, anxiety, depression)	48.5% high dose; 14.7% low dose	Resolve within days to weeks	E7 [11]; class data [2,32]
Peripheral neuropathy	27.3% high dose; 2.9% low dose; 0/6 assessed	Resolves over weeks to months; rare residuum	E7 [11]; E8 [16]; class data [49]
Hepatic enzyme elevation	15.2%/14.7%; 3/9 in the trial	Normalizes on discontinuation	E7 [11]; E8 [16,29]
Serious hepatic event requiring hospitalization	1/9	Full recovery	E8 [16,29,31]
Jarisch-Herxheimer-like reaction	Not quantified	Managed by dose reduction	E7 [11]
Syncope or near-syncope	3 individual cases	Treatment discontinued	E5 [44]; E7 [11]
Any patient-reported toxicity	13/16	Index case severe and persistent	E6 [45]
DSF-ethanol reaction (inadvertent exposure)	Not quantified in Lyme cohorts	Ranges from mild to life-threatening	[2,14]
CYP-mediated interaction (warfarin, phenytoin)		Requires dose review before initiation	[2,16]

5.18. DSF-Related Adverse Events Reported in Patients with Lyme Disease

5.18.1. General Data from Literature

Independent of the Lyme indication, class hazards directly impact this population: DSF-ethanol reactions can be caused by hand sanitizers, hygiene products, cooking ingredients, and formulations of acetaminophen, diphenhydramine, or sertraline. Inhibition of hepatic cytochrome P450 reductase may lead to toxic levels of warfarin and phenytoin (Table 3). Rarely, liver failure, cardiac toxicity, psychosis, and peripheral or central neurotoxicity have been reported even without alcohol exposure. National guidelines recommend DSF as a second-line treatment for alcohol use disorder, suitable only for patients who understand the alcohol-interaction risks and have no contraindications. Monitoring includes liver function tests every two weeks, as enzyme levels can increase rapidly, especially above 250 mg daily.

5.18.2. A Case Report with Fatal Outcome

One fatal outcome has been reported. Frankl et al. described a middle-aged man treated for chronic Lyme disease with DSF 375 mg daily, minocycline 100 mg twice daily, and tinidazole 500 mg twice daily; his wife reported he took all three at higher-than-prescribed doses [103]. This is the only death in the human evidence reviewed.

i. At presentation in the Emergency Care Unit, the patient had altered mental status, seizure-like activity, severe but rapidly resolving anion-gap acidosis (pH 7.18; lactate 7.3 mmol/L), acellular cerebrospinal fluid, and generalized EEG slowing. MRI showed symmetric diffusion restriction in both posterior putamina and medial thalamic T2 hyperintensity.
ii. On day 2, he became obtunded and was intubated. Despite no sedation, he remained difficult to arouse as imaging showed marked progression.
iii. After two and a half months of ventilator-dependent respiratory failure, severe sensorimotor polyneuropathy, depressed consciousness, and recurrent hospital-acquired infections, the patient died.
iv. Extensive testing excluded infectious, autoimmune, paraneoplastic, vasculitic, heavy-metal, and nutritional causes; serum and urine copper were normal. Negative serum Lyme antibodies did not support treatment, and the report is indexed as diagnostic error and inappropriate prescribing.
v. Two findings support DSF toxicity: imaging matched prior cases and differed from nitroimidazole toxicity, which typically affects the cerebellar dentate nuclei, caudate, and splenium; and he had been hospitalized with peripheral neuropathy soon after starting DSF eight months earlier. Electromyography in both admissions showed diffuse axonal sensorimotor polyneuropathy consistent with DSF toxicity.
vi. Three important aspects need to be considered, though neither removes the signal:
    - First, the patient took two other associated drugs, preventing isolation of DSF's effects. Tinidazole is a nitroimidazole, and its combination with DSF is a recognized cause of encephalopathy and psychosis that is generally discouraged; thus, the co-medication is part of the hazard, not merely a confounder.
    - Second, he exceeded prescribed doses, so this is not a clean test of the prescribed regimen, although self-escalation is foreseeable with unmonitored off-label use.
    - Third, one case cannot establish a rate: the point estimate is 1/1, with a 95% confidence interval of 20.7–100%, which says nothing about frequency.

This clinical case reports that the harm induced by DSF in this context can include death, in a patient taking the drug for the same off-label reason as those in [11,44], and [45], and within the same prescribing pattern.

5.19. Risk of Bias and Certainty of Evidence

Table 13 summarizes the per-study risk-of-bias analysis and the resulting certainty rating for each outcome.

Table 13. Risk-of-bias appraisal and certainty of evidence assessed by GRADE-style certainty summary.

Study	Principal Threats	Overall Appreciation	Reference
E1	Screening assay cannot distinguish inhibition from killing; single laboratory; no subculture confirmation	Moderate concerns	[4]
E2	Same laboratory lineage as E1; non-physiological intraperitoneal dose; immunological endpoints confound microbiological interpretation	Moderate to serious concerns	[79]
E3	Single laboratory; fixed concentration testing; limited strain range	Low to moderate concerns	[82]
E4	DSF one of many arms; not powered for DSF-specific inference	Low to moderate concerns	[84]
E5	No control, no blinding, selected cases, treating clinician as assessors, arbitrary dose and duration	Critical	[44]
E6	Recruitment via a call for toxicity reports (selection toward harm); diagnoses and responses unverified; no clinical relationship with respondents	Critical for benefit, serious for harm	[45]

Table 13. *Cont.*

Study	Principal Threats	Overall Appreciation	Reference
E7	No control or blinding; unstandardized outcome ascertainment; clinician-assessor; authors acknowledge placebo effect and mutual expectation bias; index cases of E5 re-included	Critical	[11]
E8	No placebo-control arm for efficacy inference; $n = 9$; early termination; ~1% randomization limits external validity; strong social-media-driven expectancy; stringent exclusions limit safety generalizability	Low for safety, serious for efficacy (imprecision and indirectness)	[16]
E9	Single uncontrolled case; concomitant minocycline and tinidazole, and doses above those prescribed, so DSF cannot be isolated. Attribution is supported by an imaging pattern typical of DSF and atypical of nitroimidazole toxicity, and by a prior DSF-associated polyneuropathy in the same patient. Published as a letter.	Critical for causal attribution; informative for hazard	[103]

Outcome	Evidence	Downgrades	Certainty	Interpretation	References
Symptom reduction in persistent Lyme disease	E5, E6, E7, E8	Risk of bias, imprecision, publication and expectancy bias, species indirectness	Very low	A benefit signal exists but is indistinguishable from placebo, regression to the mean and expectancy	[11,16,44,45]
Microbiological eradication in humans	None	No study measured a microbiological endpoint in patients	Very low (no direct evidence)	Unproven	—
Enduring remission at >=6 months	E7 only	Critical risk of bias, single center, single assessor	Very low	Hypothesis-generating only	[11]
Dose-dependent adverse events	E5, E6, E7, E8, E9 plus 70 years of class data	Indirectness of some class data	Moderate	Established and dose-related	[2,11,14,16,32,44,45,103]
Hepatotoxicity requiring discontinuation	E7, E8 plus class literature	Imprecision	Moderate	Established; mandates scheduled monitoring	[11,16,29]
Serious and fatal neurological toxicity	E9 plus class literature	Risk of bias; imprecision; confounding by co-medication	Low	A fatal outcome has been reported in a patient treated for chronic Lyme disease; attribution to DSF alone is not established	[103]
Superiority to standard antibiotics	E3, E4 (preclinical only)	Indirectness of model and species	Low	Preclinical evidence points against superiority	[82,84]
Applicability to European genospecies (*B. afzelii*, *B. garinii*)	None	No study of any genospecies other than *B. burgdorferi sensu stricto*	Very low (no direct evidence)	Entire evidence base is indirect for European and Romanian Lyme borreliosis	[54,65,105,106]

Certainty for benefit is very low in each outcome, whereas certainty for dose-dependent harm is moderate; the evidence base is therefore substantially more informative about what DSF does to patients than about what it does to the organism. The species-indirectness downgrade applies across literature and cannot be recovered by any future human trial conducted only in North America.

Table 14 sets out the reasoning behind every certainty rating, including the starting certainty for each study design and the reason for each downgrade. Detailed analysis with supporting quotations from each source is given in Supplementary Table S3.

Table 14. GRADE assessment. Each outcome begins at the certainty appropriate to its contributing study designs and is downgraded explicitly. "Serious" denotes a one-level downgrade and "very serious" a two-level downgrade. Species indirectness denotes the absence of any evidence for genospecies other than *B. burgdorferi* sensu stricto.

Outcome	Contributing Studies	Starting Certainty	Risk of Bias	Inconsistency	Indirectness	Imprecision	Publication Bias	Final Certainty
Symptom reduction in persistent Lyme disease	E5–E8 [11,16,44,45]	Low (observational + 1 pilot RCT)	Very serious (−2): no control, unblinded, clinician-assessed outcomes	Serious (−1): dose–response direction conflicts between [11] and [16]	Serious (−1): species indirectness; surrogate symptom endpoints only	Serious (−1): all CIs span clinically important and null effects (Table 7)	Suspected: field with strong patient-community engagement	Very low
Microbiological eradication in humans	None	Not applicable	Not assessable	Not assessable	Not assessable	Not assessable	Not assessable	Very low (no direct evidence)
Enduring remission >=6 months	E7 [11]	Low (single observational series)	Very serious (−2): single practice, single assessor, post hoc dose strata	Not assessable (single study)	Serious (−1): species indirectness	Serious (−1): 36.4%, 95% CI 22.2–53.4 from a non-randomized comparison	Suspected	Very low
Dose-dependent adverse events	E5–E9 [11,16,44,45,103] + 70 years of class data [30,47–51]	Moderate (consistent across designs and indications)	Serious (−1): ascertainment differs across sources	Not serious: direction consistent in every source	Not serious: harms are drug-level, not disease-level	Not serious for common events	Not suspected for harms	Moderate
Hepatotoxicity requiring discontinuation	E7, E8 [11,16] + class literature [29,31,48]	Moderate	Serious (−1)	Not serious	Not serious	Serious (−1): 3/9 in [16], 95% CI 12.1–64.6	Not suspected	Moderate
Serious and fatal neurological toxicity	E9 [103] + class literature [49,51]	Low (single case report)	Serious (−1): single uncontrolled case; concomitant minocycline and tinidazole; no rechallenge	Not assessable (single study)	Not serious: the patient was treated for chronic Lyme disease with DSF	Serious (−1): 1/1, 95% CI 20.7–100	Not suspected: fatal outcomes are unlikely to go unreported	Low
Superiority to standard or candidate antibiotics	E3, E4 [82,84]	Low (preclinical only)	Not serious	Not serious: both independent studies agree	Very serious (−2): in vitro and murine surrogates for human efficacy	Not serious	Not suspected	Low (evidence points against superiority)
Applicability to European genospecies	None	Not applicable	-	-	Very serious (−2): no study of *B. afzelii* or *B. garinii*	-	-	Very low (no direct evidence)

5.20. Discussions

5.20.1. Disulfiram vs. Other Candidates for Lyme Disease Therapy

Before those findings, DSF's standing among the agents surveyed in Section 5.6 can now be stated, because it depends on the evidence just presented:

i. Disulfiram is the only agent among them to have undergone a randomized human trial, a notable but limited distinction, and that trial stopped early [16].

ii. Preclinical data do not support DSF as the leading candidate: hygromycin A provides selective activity with microbiome sparing [80], azlocillin kills doxycycline-tolerant persisters and is effective in mice [81], and nitroxoline combinations outperform DSF in comparable assays [82,83].

iii. The two agents with the largest uncontrolled human datasets, DSF and dapsone, rest on retrospective series that are unblinded and clinician-assessed, with substantial discontinuation for adverse effects [11,86–88]; this is a limitation of the field rather than of DSF alone.

5.20.2. Findings About DSF in Lyme Disease

Several main findings emerge from the current synthesis:

a. First, the preclinical evidence is weaker than the clinical use suggests. The two studies showing strong activity come from a single laboratory lineage and use an ATP-based viability test that cannot distinguish between inhibition and cell killing [4,79]. An independent study using a more rigorous subculture regrowth test found DSF less effective than cefuroxime, clarithromycin, and nitroxoline, both alone and in combination [82]. The only in vivo eradication evidence involved doses about fifteen times higher than the clinical target [11,79].

b. Second, the strongest claim in this literature—the dose–response relationship for lasting remission—is the most uncertain [11]. It is based on a single, unblinded practice without controls or standardized measures, with clinicians as outcome assessors. The authors noted potential placebo effects and expectation bias [11]. A small, randomized pilot study found responders at both low and high doses after just three to six weeks of treatment [16], which directly challenges the logic that higher doses and longer durations are necessary, a common approach in high-risk prescribing [11,44].

c. Third, the harm signal remains the most dependable element of the evidence. It is consistent across an unblinded series favorable to the drug [11], a harm-oriented survey [45], and a monitored prospective trial [16]: hepatic enzyme elevation, psychiatric disturbances, fatigue, and peripheral neuropathy—all dose-dependent and mostly reversible, with at least one serious hepatic event per nine monitored participants [11,16]. This pattern aligns with 70 years of literature on hepatotoxicity and neurotoxicity at 250–500 mg/day [2,29,32]. Even in a trial excluding cardiac, hepatic, renal, neurological, and psychiatric comorbidities, five of nine participants still discontinued treatment [16].

The feasibility finding warrants particular attention. Randomizing just 1% of those screened, with inadequate serological evidence as the primary reason for exclusion [16], shows that a typical monotherapy trial with strict diagnostic criteria is logistically nearly impossible. Possible solutions include prospective stratification based on diagnostic certainty and broadening, yet transparently classifying, entry criteria [56], which would trade some internal validity for increased external validity in a controlled manner.

d. A fourth, often overlooked constraint is taxonomic; literature frequently uses Lyme disease and Lyme borreliosis interchangeably. All reviewed in vitro and animal studies used *B. burgdorferi sensu stricto*, almost always the B31 reference strain. Three of the four human reports originate in the United States [11,16,44]; the fourth is a French patient-organization survey [45], and is therefore European in origin, although its respondents were treated on the basis of the same United States-derived evidence, and no infecting genospecies was identified in any human report.

e. In Europe, especially Romania, *B. afzelii* is the most prevalent genospecies, followed by *B. garinii*, with *B. burgdorferi* sensu stricto being rare [65,105,107]. These genospecies differ phylogenetically and in tissue tropism—*B. afzelii* is linked to skin disease, and *B. garinii* to neuroborreliosis [54]. They also differ in susceptibility-related biology, including outer-surface protein expression, plasmid content, and complement resistance, so in vitro activity against B31 cannot be assumed to apply to *B. afzelii* or *B. garinii*. No studies have tested DSF on these genospecies. For European clinicians, this means the evidence base is indirect both biologically and methodologically, and the certainty of benefit is even lower than the very low rating based on study design alone.

5.21. Research Gaps

- No human study has employed direct detection methods, such as culture or biomarkers; all outcomes rely on self-report or clinician impression.
- The immunosuppressive effects observed in mice have not been examined as mediators of symptom change in humans, making it unclear whether antimicrobial or immunomodulatory mechanisms are responsible.
- There is no human pharmacokinetic data linking plasma DSF or diethyldithiocarbamate levels to the non-linear activity observed in vitro, so the relevant exposure window in clinical settings remains unknown.
- The dose–response relationship for therapeutic benefit is inconsistent between the two largest human datasets and remains unresolved.
- No clinical study has evaluated DSF as a combination adjunct. The only combination data are in vitro and are unfavorable: cefuroxime plus DSF left 41.7% of stationary-phase organisms viable, against 25.9% for cefuroxime plus clarithromycin and 27.5% for cefuroxime plus nitroxoline, and DSF-containing three-drug combinations failed to prevent subculture regrowth [82]. Combination pharmacology remains the direction current persister research favors [83–85], and no DSF-containing regimen has been tested in an animal model of persistent infection.
- DSF has not been tested against *B. afzelii*, *B. garinii*, or any genospecies beyond *B. burgdorferi sensu stricto*, leaving European and Romanian disease contexts uninvestigated; initial in vitro testing against European clinical isolates would be a minimal step.
- Reports of activity against *Babesia* and *Bartonella* are based solely on uncontrolled clinical observations without supporting in vitro data.
- Formulation work has begun to address the bioavailability and toxicity constraints identified in this review but has not yet reached any biological endpoint. A low-dose DSF rectal suppository using a polyethylene glycol and hydroxypropyl-β-cyclodextrin matrix has been developed and characterized to avoid hepatic first-pass metabolism and limit dose-limiting toxicity in post-treatment Lyme disease syndrome [108]. The work reports dissolution and permeation data only, with no *Borrelia* and no patients, so it was not eligible for the synthesis; it is noted here because it addresses precisely the gap between in vitro potency and tolerable human exposure that this review identifies.

5.22. Reactive Sulfur Species and Hydrogen Sulfide Signaling: An Untested Pathway

One mechanistic dimension missing from the DSF literature on Lyme borreliosis, and raised during peer review, is the role of reactive sulfur species. DSF is a sulfur-rich molecule that degrades to carbon disulfide, which has been measured in the blood of treated patients [8]. More broadly, dithiocarbamates are part of the chemistry that generates carbonyl sulfide and hydrogen sulfide (H_2S) in biological systems [109]. None of the studies reviewed here measured sulfur species except as toxicological endpoints, so the discussion that follows presents a hypothesis, not a finding.

This hypothesis is worth highlighting because H_2S has well-described, opposing roles on both sides of this host–pathogen relationship. In the host, H_2S and protein persulfidation help regulate redox balance, mitochondrial bioenergetics, and inflammatory signaling, and changes in H_2S tone have been described in inflammatory and degenerative disease [109]. Several effects attributed to DSF in the murine Borrelia model—reduced inflammatory marker expression and altered antibody and lymphocyte profiles [79]—could plausibly result from disrupted sulfur signaling, even without any antimicrobial action. This places the mechanism firmly in the host-directed column of Figure 3.

On the bacterial side, the evidence is contested, making the question testable. Endogenous H_2S was initially proposed as a universal bacterial defense that limits reactive oxygen

species and enhances antioxidant enzymes, thereby conferring tolerance to several antibiotic classes [110]. More recent work, however, has shown the opposite in organisms lacking the biosynthetic pathway. In *Acinetobacter baumannii*, which lacks cystathionine-beta-synthase, cystathionine-gamma-lyase, and 3-mercaptopyruvate sulfur transferase, exogenous H_2S sensitized the organism to gentamicin, colistin, rifampicin, and clarithromycin, and reversed acquired gentamicin resistance by shifting it toward a pro-oxidant state [111]. To our knowledge, whether *B. burgdorferi* has a functional H_2S-generating pathway remains unknown. The organism is already unusual in its sulfur and metal biology, substituting manganese for iron and relying on coenzyme A disulfide reductase rather than a glutathione system [15], making this question meaningful.

Further research could interrogate the *B. burgdorferi* genome and transcriptome for homologs of the three H_2S-generating enzymes and measure H_2S production directly in culture. It could also determine whether DSF exposure alters sulfur-species flux in the spirochaete, which would distinguish a sulfur-mediated mechanism from the metal-chelation and thiol-alkylation mechanisms currently proposed. Additionally, it could test whether exogenous H_2S donors or inhibitors shift the minimum bactericidal concentration of DSF and of the standard Lyme antibiotics. A positive result in this last suggested study would be of immediate practical interest, since it would identify a modifiable determinant of antibiotic tolerance in an organism whose persister forms are the central problem in this field.

6. Essential Considerations

6.1. The Strengths of the Present Review

The present work has several distinct strengths:

i. It integrates DSF pharmacology, cross-indication evidence, and Lyme literature into a single framework. It also describes in detail all mechanistic pathways and molecular targets involved in DSF therapeutic benefits and toxic effects. Typically, DSF is reviewed within isolated fields, which obscures the overarching pattern across disciplines: detailed mechanistic activity, followed by limited or inconsistent controlled trial results, and, in the only well-powered randomized comparison against standard care, no benefit was observed, coupled with significantly increased toxicity [34,35].

ii. It clearly considers DSF as a prodrug and structures the mechanistic explanation around the species present in tissue, not just the parent molecule. Presenting the metabolic cascade before the target list clarifies why oral dosing is an imperfect indicator of target-site exposure and why dose–response relationships in any indication require pharmacokinetic evidence, which has rarely been provided [6,7,9,10].

iii. DSF toxicity is assessed at the molecular target level rather than at the clinical syndrome level. This analysis reveals two often overlooked points: first, that NPL4 and proteasome inhibition, along with gasdermin D blockade, serve as both therapeutic and toxic mechanisms, meaning no dose can separate their effects; and second, that monitoring should be tailored to the specific target rather than applied uniformly [3,5,17,28,35,36].

iv. The DSF use in Lyme borreliosis section employs appropriate risk-of-bias domains and a GRADE-style certainty assessment across all nine primary studies. This produces conclusions that are explicit enough to be challenged and highlights a previously unmentioned species-indirectness issue: all preclinical work involves *B. burgdorferi sensu stricto*, and no human report identified the infecting genospecies, so no study anywhere in this literature provides direct evidence for the genospecies that dominate European disease [4,54,65,79,82,84,105,107]. It also shows that current studies cannot distinguish the antimicrobial and immunomodulatory hypotheses, since no human study has assessed microbiological endpoints [11,16,44,45].

v. DSF pharmacotoxicology is explicitly used to argue for detectability. Serious adverse events occur at rates of about 1 in 10,000 to 1 in 30,000 treated patients annually, so in the 93 exposed patients with Lyme borreliosis identified here, the available studies cannot reliably detect such events; their absence in reports is uninformative rather than reassuring [30,47–49]. Throughout, we analyzed uncontrolled clinical series as data, acknowledged their design flaws, and applied the same rigorous standards to trials that produced conflicting results [11,16,86–88].

vi. Each proportion reported by a human study was re-expressed with its denominator and a 95% confidence interval, computed by the Wilson score method from the published numerators and denominators because the source publications reported bare percentages. This makes the imprecision visible rather than implicit: enduring remission on the low-dose regimen, for example, is 0/34, 0% (95% CI 0–10.2), and the fatal case is 1/1, 100% (95% CI 20.7–100), a figure that carries no information about frequency at all. The same arithmetic resolved a double-counting that circulates in summary accounts of this literature: the four effectiveness reports contain 96 participant entries but 93 unique individuals, because the three index cases of [44] are identified in [11] as Cases 18, 49 and 10 of that series.

vii. The review brings into this indication a documented fatal outcome that none of the clinical reports or narrative accounts assembled here had carried forward [103], together with the mechanism that makes it more than an isolated misfortune: the patient received tinidazole alongside DSF, and the DSF-nitroimidazole combination is a recognized cause of encephalopathy and psychosis. Because that pairing reflects a real prescribing pattern rather than an experimental error, it converts a general warning about class toxicity into a specific, checkable contraindication.

viii. We detailed the systematic component; every search string, the interface used, the date of execution, and the per-source yield are given in Supplementary Table S1; every count in the flow diagram is an exact integer traceable to the deduplicated library; and risk-of-bias and certainty judgments are recorded domain by domain in Table 13 and Supplementary Table S3, with the reason for each downgrade stated. A reader who disagrees with a rating can see precisely which judgment to dispute, which is the practical test of a transparent appraisal.

6.2. Limitations

Our comprehensive review has a hybrid structure, and several limitations warrant mention.

6.2.1. Narrative Component

Sections 2–4 were not searched systematically; the indications and mechanisms covered reflect judgment rather than a protocol. Table 2 reports the highest level of evidence in each area, not a complete account. The survey of competing agents in Section 5.6 is likewise narrative, and the comparative judgment it supports is given in Section 5.20.

6.2.2. No Registration

No protocol was registered or deposited, so the pre-specification of eligibility criteria, search strategy and extraction fields rests on this manuscript alone and cannot be checked against a time-stamped record.

6.2.3. Single-Author Search, Select and Report

One researcher performed screening, extraction and risk-of-bias assessment; single screening misses eligible records more often than duplicate screening [93]. Searching, selection and transcription were each carried out twice, and every extracted value was

checked against its source, which addresses mis-transmitted data but not mistaken judgment: two passes by one reader cannot detect a consistent error, and no agreement statistic applies.

6.2.4. Coverage

The search covered six sources—PubMed, Europe PMC, the Cochrane Library including CENTRAL, bioRxiv, medRxiv and ClinicalTrials.gov—together with forward and backward citation chasing and a publisher-platform redundancy check. Embase, Scopus and Web of Science were not searched. Europe PMC offsets this in part, since it indexes deposited full text and preprints and returned every PubMed record plus 159 others; the residual risk is nevertheless demonstrable, in that the foundational in vitro screening study [4] names the drug nowhere in its title or abstract and entered this review by citation chasing alone. Grey literature beyond preprints and registered trials was not exhaustively retrieved.

6.2.5. No Pooling

Heterogeneity in design, population definition, dosing, and outcome instruments precluded meta-analysis [11,16,44,45]; the confidence intervals in Table 8 describe the precision of single studies and are not pooled estimates. Publication bias in a field with strong patient-community engagement probably favors positive reports.

6.2.6. Unverified Mechanisms

Mechanistic hypotheses from in vitro and animal work are reported as published and were not independently verified; several, including biofilm penetration and the sulfoxide versus sulfone question in ALDH inhibition, remain disputed.

6.2.7. Shelf Life and Scope

Toxicology and evidence levels in oncology and metabolic research move faster than pharmacology, so those sections will date first. This review appraises evidence rather than individual cases: it states what the evidence supports, not what any clinician should do for a particular patient.

6.3. Potential Applications

The value of this review lies less in any single finding than in what becomes visible once the evidence is assembled in one place and appraised to a common standard. Five uses follow directly from it.

6.3.1. For Clinicians Asked to Prescribe DSF in Lyme Borreliosis

The review states what the evidence supports and what it does not, and it supplies the two facts most often missing from that conversation: that no human study has ever measured whether DSF clears the organism, and that an absence of serious adverse events across 93 exposed patients is uninformative rather than reassuring, because events occurring at 1 in 10,000 to 1 in 30,000 per year cannot appear in a series of that size. The target-level toxicology in Table 3 turns generic caution into specific monitoring, and the documented DSF-nitroimidazole hazard into a checkable contraindication.

6.3.2. For the Design of a Decisive Trial

The review specifies what such a trial must do rather than merely calling for one: measure a microbiological or biomarker endpoint, because symptomatic endpoints alone cannot separate spirochaete killing from anti-inflammatory action; identify the infecting genospecies; pre-specify diagnostic certainty, since inadequate serological evidence was the main reason only about 1% of those screened were randomized in the one completed

trial [16]; and keep exposure short and low-dose, given that no dose separates benefit from harm.

6.3.3. For European and Romanian Practice

The species-indirectness finding is the most transferable result here. Every preclinical study used B. burgdorferi sensu stricto and no human report identified the infecting genospecies, so the entire evidence base is indirect for the *B. afzelii*- and *B. garinii*-dominated disease of Europe. In vitro testing against European clinical isolates is a small, inexpensive study that would close a gap no larger trial can close.

6.3.4. For Drug-Repurposing Methodology

Disulfiram is an unusually complete worked example of a pattern that recurs across repurposing: broad mechanistic activity, enthusiastic early-phase reporting, and failure to translate when finally tested against standard care. Framing the account around the prodrug and the species actually present in tissue, rather than the parent molecule, transfers to any candidate whose activity depends on metabolites; the same applies to appraising toxicity at the molecular target level rather than the clinical syndrome.

6.3.5. For Formulation and Future Mechanistic Work

The review identifies the precise gap further development must close: the distance between in vitro potency and tolerable human exposure; early formulation work directed at that gap has begun [108] but has reached no biological endpoint. Section 5.22 adds a reactive sulfur species hypothesis with three falsifiable experiments, so it can be tested or discarded rather than repeated.

7. Conclusions

Disulfiram is a prodrug with broad mechanistic pathways and numerous molecular targets. Its metabolites chelate copper, zinc, and manganese, and modify reactive cysteines. Through these mechanisms, it interacts with at least twelve targets related to protein turnover, epigenetic regulation, inflammatory cell death, chemokine signaling, catecholamine synthesis, and hepatic drug metabolism. This broad target range supports its consideration for many uses but also makes it challenging to demonstrate clinical specificity. The repurposing literature consistently shows a pattern: strong preclinical activity, small or inconsistent controlled results, and in the few well-powered randomized trials comparing it to standard care, no clear benefit and increased toxicity. Only alcohol use disorder has regulatory support, and even then DSF is considered a second-line treatment.

Disulfiram was introduced to treat Lyme borreliosis based on limited evidence, including a single in vitro test and three case reports, with no placebo trials. Preclinical studies show it performs worse than standard and experimental antibiotics against persister forms, and it was ineffective as a sole treatment in animal models of persistent infection. Human data include one uncontrolled report indicating high benefit and toxicity, a survey mainly reporting toxicity, and a small randomized pilot that was stopped early for four stated reasons—end of project funding, pandemic-related enrolment delay, higher-than-expected adverse events, and sufficiency of the information gathered—in which five of nine participants discontinued study medication. The certainty of its benefit is very low, especially outside North America, as only *B. burgdorferi sensu stricto* has been tested, leaving indirect evidence for European strains like *B. afzelii* and *B. garinii*.

The evidence for dose-dependent harm is moderate, and the harm reported in this indication now extends to a fatal outcome: one patient treated for chronic Lyme disease with disulfiram, minocycline and tinidazole died after chemically induced seizures, impaired

consciousness and acidosis. A single confounded case establishes no rate, but it fixes the upper bound of what has actually happened to a patient taking this drug for this indication. No professional guidelines endorse disulfiram for Lyme disease, and it remains an off-label, unproven treatment. When used, the recommended approach is to administer the lowest effective dose for no longer than six to eight weeks, perform liver function tests every two weeks, exclude patients with cardiac, liver, psychiatric, or nerve conditions, provide thorough counseling on accidental alcohol exposure, and review cytochrome P450 interactions.

Further research urgently needs to conduct a placebo-controlled trial with clearly defined diagnostic certainty, short low-dose treatment, and at least one objective or biomarker outcome.

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Abbreviations

The following abbreviations are used in this manuscript:

20S proteasome	Proteolytic core particle of the proteasome
AAA	ATPase associated with diverse cellular activities
AAN	American Academy of Neurology
ACR	American College of Rheumatology
ALDH	Aldehyde dehydrogenase
ALDH1A1, ALDH2	Aldehyde dehydrogenase isoforms 1A1 and 2
APC	Adenomatous polyposis coli, a tumor-suppressor gene
ATF/CREB	Activating transcription factor/cyclic AMP-responsive element-binding protein
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
B31	Reference strain of *Borrelia burgdorferi sensu stricto*
BmtA	Borrelia metal transporter A
C3H/HeN	Inbred laboratory mouse strain
CCR2, CCR5	C-C chemokine receptor types 2 and 5
CD3, CD4, CD14, CD19, CD45	Cluster of differentiation cell-surface antigens
CENTRAL	Cochrane Central Register of Controlled Trials
CGI-I	Clinical Global Impressions-Improvement

CI	Confidence interval
CoADR	Coenzyme A disulfide reductase
COVID-19	Coronavirus disease 2019
CS_2	Carbon disulfide
CuET	Bis(diethyldithiocarbamate)-copper (II)
CYP	Cytochrome P450
Cys	Cysteine
DDC	Diethyldithiocarbamate
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNMT1	DNA methyltransferase 1
DSF	Disulfiram
E1-E9	Identifiers of the nine studies included in the systematic review (Table 6)
EUCALB	European Concerted Action on Lyme Borreliosis
FDA	United States Food and Drug Administration
FROUNT	Cytosolic protein that binds the chemokine receptors CCR2 and CCR5
GRADE	Grading of Recommendations Assessment, Development and Evaluation
GSDMD	Gasdermin D, the pore-forming executioner protein of pyroptosis
GSQ-30	General Symptom Questionnaire-30
H1-H5	Hypotheses advanced for disulfiram benefit in Lyme borreliosis (Section 5.5)
H_2S	Hydrogen sulfide
HIV	Human immunodeficiency virus
IDSA	Infectious Diseases Society of America
IgG, IgG2b, IgM	Immunoglobulin G, immunoglobulin G2b and immunoglobulin M
IL-1β	Interleukin-1 beta
MBC	Minimum bactericidal concentration
MCID	Minimal clinically important difference
MCS	Mental Health Component Summary
MDA-MB-231	Human triple-negative breast cancer cell line
MDPI	Multidisciplinary Digital Publishing Institute
MeDDC	S-methyl-N,N-diethyldithiocarbamate
MEDLINE	Medical Literature Analysis and Retrieval System Online
MERS-CoV	Middle East respiratory syndrome coronavirus
MeSH	Medical Subject Headings
MMP-2, MMP-9	Matrix metalloproteinase-2 and matrix metalloproteinase-9
Nanog, Oct4	Transcription factors associated with the stem-like cell state
NCT	ClinicalTrials.gov registry identifier
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NPL4	Nuclear protein localization protein 4, cofactor of p97
NSCLC	Non-small-cell lung cancer
NTSS-6	Neuropathy Total Symptom Score-6
OspA	Outer surface protein A
p97	Hexameric AAA-family ATPase, also known as valosin-containing protein
PCR	Polymerase chain reaction
PCS	Physical Component Summary
PD-L1	Programmed death-ligand 1
PI	Propidium iodide
PMC	PubMed Central
POH1	Proteasomal deubiquitinase POH1
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PRISMA-S	PRISMA extension for reporting literature searches
PROMIS-29 SPADE	Patient-Reported Outcomes Measurement Information System 29-item profile; SPADE denotes the symptom cluster of Sleep disturbance, Pain, Anxiety, Depression and low Energy/fatigue
PTLDS	Post-treatment Lyme disease syndrome
Q-LES-Q	Quality of Life Enjoyment and Satisfaction Questionnaire
RCT	Randomized controlled trial
RoB 2	Revised Cochrane risk-of-bias tool for randomized trials
ROBINS-I	Risk Of Bias In Non-randomized Studies of Interventions
ROS	Reactive oxygen species
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
Sensu lato	In a broad sense
Sensu stricto	In a narrow sense

SF-36	36-Item Short Form Health Survey
SWiM	Synthesis Without Meta-analysis
SYBR Green I	Fluorescent nucleic acid stain used in viability assays
SYRCLE	Systematic Review Center for Laboratory Animal Experimentation
TNSc	Total Neuropathy Score, clinical version

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